Comprehensive characterization of chemical structures in heavy crude oil asphaltenes by using liquid chromatography and ultrahigh-resolution mass spectrometry

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"Life is like a game of chess. To win you have to make a move. Knowing which move to make comes with IN-SIGHT and knowledge, and by learning the lessons that are accumulated along the way. We become each and every piece within the game called life!"

Allan Rufus, The Master's Sacred Knowledge

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ABSTRACT

Crude oil is known as the main global energy source, however the reserves are limited. As the remaining valuable light oils are diminishing, alternative solutions such as upgrading of high boiling conventional and unconventional oil sources such as tar sands and oil shales have to be exploited for replacement to overcome the worlds emerging energy needs. These alternative sources contain a high amount of heteroatoms (e.g. N, O, S) and are of high viscosity causing difficulties to the environment and on industrial sites during oil transportation and production. Most of the problems are associated with asphaltenes which are the heaviest fraction of crude oil. Asphaltenes are considered to be responsible for clogging pipelines and wellbores, creating deposits. A thorough understanding of the chemical and physical attributes of asphaltenes is essential to contribute to optimization, improvements and new technological developments in up-to-date refining processes.

The extreme complexity of crude oil consisting of probably more than one million different chemical compounds leads to a broad range of properties in terms of polarity, molecular weights and molecular architecture. In order to receive a more clear and comprehensive picture of structural details, different approaches, meaning combinations of advanced analytical techniques, need to be involved. Mass spectrometry with ultrahigh resolution and mass accuracy provides information on a molecular level. One of the main objectives of this study is the structural elucidation of individual compounds in very complex oil mixtures using MS/MS measurements as the method of choice. Unfortunately, isolation of small mass windows leads to a large number of different compounds that would participate in the fragmentation process leading to MS/MS spectra that are extremely difficult to interpret. Therefore, prior simplification is required to reduce the different types of species within the selected mass window subjected to fragmentation. Application of two different chromatographic separation methods, each focusing on different attributes of the compounds, is beneficial prior to mass spectrometric analysis. The separation methods applied here are size exclusion chromatography (separation according to molecular size) and argentation chromatography (separation according to unsaturation and heteroatom content). The combination of these separation methods makes it possible to apply collision activated

dissociation methods to study structural details and suggest a theory of asphaltene structures and its formation.

KURZFASSUNG

Erdöl ist bekanntermaßen der weltweit wichtigste Energieträger, wobei jedoch die Reserven begrenzt sind. Mit zunehmendem Verbrauch wertvoller, leichter Öle müssen alternative Strategien gefunden werden, um den weltweit steigenden Energiebedarf zu decken, wie etwa die Aufwertung konventioneller und unkonventioneller schwerer Ölquellen wie Ölsande oder Ölschiefer. Mit diesen alternativen Quellen geht gemeinhin ein erhöhter Heteroatomgehalt (z.B. N, O, S) einher, was neben einer erhöhten Viskosität auch zu weiteren Problemen während Transport und Prozessierung in der Umwelt oder in Industrieanlagen führt. Der Großteil dieser Probleme wird mit der schwersten Fraktion des Erdöls, den Asphaltenen, in Verbindung gebracht. Diese zeichnen verantwortlich für ein Verstopfen von Pipelines und Bohrlöchern, indem sie Ablagerungen bilden. Ein tiefgreifendes Verständnis der physiko-chemischen Eigenschaften von Asphaltenen ist unerlässlich, um neue Technologien und Prozessoptimierungen zu entwickeln, die den Raffinerieprozess verbessern.

Die extrem hohe Komplexität von Erdöl umfasst eine breite Spanne von Eigenschaften von Polarität über molekularer Masse und Molekülstruktur. Um ein klareres und verständlicheres Bild von Strukturdetails zu erlangen müssen verschiedene Ansätze, das heißt Kombinationen verschiedener leistungsstarker analytischer Techniken, verfolgt werden. Massenspektrometrie mit ultrahoher Massenauflösung und Massengenauigkeit erlaubt es, Informationen auf molekularer Ebene zu erlangen. Für Untersuchungen der Struktur einzelner Verbindungen, die ein Hauptaspekt dieser Arbeit sind, stellen MS/MS-Messungen die Methode der Wahl dar. Eine vorhergehende Probenvereinfachung ist notwendig, um die Anzahl verschiedener Spezies im untersuchten Massenfenster zu minimieren. Die Anwendung chromatographischer Methoden, die jeweils Trennung nach verschiedenen Parametern beleuchten, wie etwa Größenausschlusschromatographie (Molekülgröße) oder silberbasierter Ligandenaustauschchromatographie (Sättigungsgrad und Heteroatomgehalt), ist Thema dieser Arbeit. Die konsekutive Nutzung dieser chromatographischen Methoden führt zu einer hinreichenden Probenvereinfachung und erlaubt somit einen detailierten Einblick in mögliche Strukturmerkmale von Asphaltenen.

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CHAPTER 1.

GENERAL INTRODUCTION

One of the biggest issues in the world is energy consumption. Conventional light and sweet crude oil as the current main global energy source are diminishing, therefore scientists and technologists are making huge efforts to find alternative solutions such as exploitation of renewable energy sources including wind or solar radiation.[1] With the current technology, however, these cover only a small part of the energy needs. While developing technological improvements, other sources such as heavy conventional and unconventional crude oils are utilized and upgraded into economically more valuable products.[2]

Conventional oils contain mostly hydrocarbons of low molecular weights which are desired for having high economic value. They have low viscosity and contain low amounts of heteroatoms (e.g. nitrogen, oxygen, sulfur) and metals in contrast to heavy and unconventional crude oils such as oil shale [3] or tar sands [4, 5] which have high viscosity, low solubility, high boiling point ranges and contain higher amounts of heteroatoms making their handling more difficult and problematic. These unfavorable properties cause additional technical problems such as precipitation and require extra considerations during transportation and production. Figure 1-1 shows an overview of the features of conventional and unconventional oils together with their technological value.[6, 7]

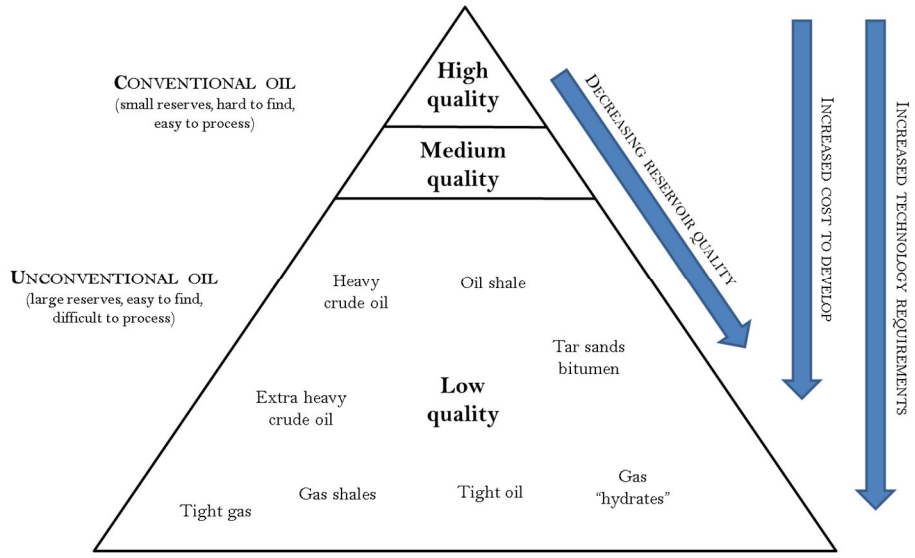

Figure 1-1. Summary of conventional and unconventional crude oil properties mostly based on Canada's hydrocarbon resources.[6]

As heavier crude oils are going to supplant light crude oils, due to their unfavorable features, technological improvements and developments of chemical processes are needed to convert them into more valuable products to produce the maximum profit. In order to make improvements and solve the accompanying problems, it is essential to know and understand the properties of unconventional crude oils. It is not enough to describe only the bulk properties but more importantly an exhaustive understanding is required on a molecular level.

Due to technological considerations, the most important bulk properties of the crude oil are the boiling point distribution, composition, density, and viscosity to evaluate the yield at the different cuts of the distillation column, to determine the paraffinic nature of the crude oil and to estimate the tendency for precipitation while oil flows through pipelines, respectively.[8, 9] The higher the boiling point, the higher molecular weights of the compounds, the larger is the amount of carbon atoms per molecule. This implies an increasing number of possibilities of different types of isomeric compounds, therefore the complexity becomes higher.[8, 10-12] Higher complexity requires the use of multiple analytical techniques, each providing specific pieces of information about the chemical composition on a molecular level.

The properties of the compounds present in complex heavy oils are very diverse and each of them can cause specific problems in the production process. The most difficult to handle and most complex fraction is considered to be the so called asphaltenes. Asphaltenes are concentrated in the highest boiling fraction or non-distillable residues, therefore they possess characteristics such as very low volatility and limited solubility which makes them difficult to analyze and limits the possible analytical techniques. Therefore, the understanding and handling of this fraction is very challenging and important at the same time.

Asphaltenes can have practical and useful applications such as being basic material for pavements and road construction, and usage as corrosion inhibitor or waterproof component of coatings. However, in the oil industry, especially in the production system, asphaltenes are very troublesome and lower the economic value of crude oil.[13] During transportation and refining processes they can precipitate in pipelines, wellbores and tubes leading to their deposition and eventual clogging which may result in reduced production. The molecular composition of asphaltenes varies and depends on several factors such as the origin of the crude oil and its maturity.[14] Although the above

mentioned problems are occurring widely, in some countries such as Canada, USA, Venezuela and Kuwait they are extremely challenging, as the presence of asphaltenes is extremely high.[15] The main and general considerations in the technical and productive systems to alleviate the potential issues are:

- prediction of potential deposits by knowing the physical and chemical properties of an oil and its asphaltene fraction;
- prevention by monitoring of facilities;
- control by application of appropriate chemical treatments; and
- remediation by removal and recovery of precipitates.[14]

To fully understand asphaltenes' behavior and their effect on oil properties, an integrated cooperation is required between scientists (chemists and geochemists) and engineers.

1.1 Asphaltene characterization

Asphaltenes have been known since the drilling of the first commercial oil wells. The term is originated from J. B. Boussingault in 1837 when he noticed these compounds in the distillation residue of bitumen.[13]

Asphaltenes cause problems such as precipitation not only at the production level but also at laboratory scale during experiments.[16] For operational concerns an in-depth knowledge of their characteristics (chemical composition, molecular weight, structure) is necessary. Asphaltenes are black or dark brown solid materials, containing high amounts of heteroatoms (e.g. nitrogen, oxygen, sulfur) and trace metals (e.g. vanadium, nickel), formed by polycondensed aromatic structures with a high number of carbon atoms (and relatively low amounts of hydrogen).[17] Their solid state imparts mainly deleterious characteristics in fluid crude oils such as low distillate yields, high viscosity and emulsion stability.[16] Apparently, asphaltene deposit in the pipelines is troublesome to the oil production. For example in the production site pressure drop can provoke flocculation that is responsible for deposit formation.[18] Besides pressure, other factors such as temperature, composition of the crude oil or the conditions inside the pipelines can influence asphaltene stability. Obviously, it becomes evident that asphaltenes in the oil field and in the laboratory implies slightly different attributes.[17]

Regarding their definition asphaltenes are the *n*-alkane (e.g. *n*-heptane or *n*-pentane) insoluble fraction of crude oil, but are soluble in aromatic solvents (e.g. toluene or benzene). They do not have a definite melting point, but decompose upon heating and result in fullerene-type compounds.[19]

Since asphaltenes are very essential components of crude oil and play an important role in production processes, an appropriate preparation method has been developed on a laboratory scale for further experiments and investigations. This method is part of the so called SARA fractionation where crude oil is separated into saturates (S), aromatics (A), resins (R) and asphaltenes (A) depending on their solubility as a first step to reduce the complexity.[9, 20-22] A schematic of the SARA fractionation procedure is shown in Figure 1-2. Asphaltenes are first removed from the other crude oil components by the addition of an *n*-alkane such as *n*-heptane. The precipitated solid sample needs to be washed very carefully to remove all of the unwanted residuals to get pure asphaltenes.[23] This is a critical step in the separation process and there are debates what the adequate procedure is e.g. regarding the washing time.[24] The non-precipitated remaining components, called maltenes, are further fractionated by open tubular chromatography on silica or neutral alumina using various solvents with increasing polarity for each fraction. There are numerous procedures, each with different combinations of solvents. As one example, *n*-heptane, toluene and a mixture of toluene and methanol (9:1, *v/v*) are used to elute saturates, aromatics and resins, successively.[21]

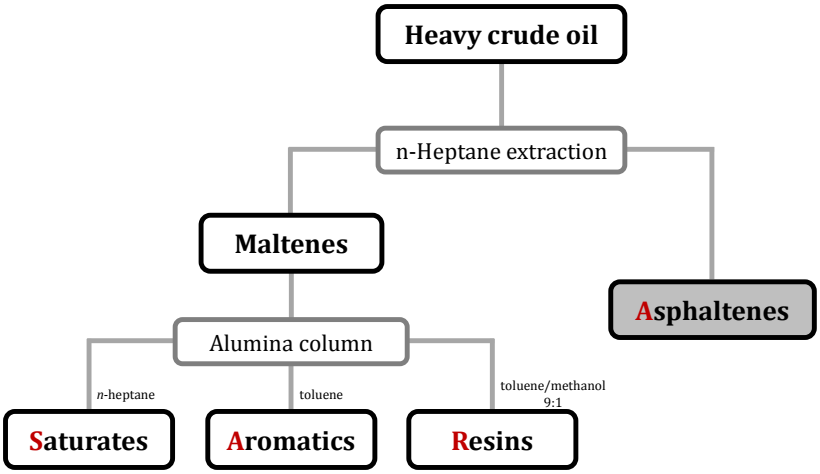

Figure 1-2. SARA fractionation procedure for the separation of heavy crude oil into fractions of saturates, aromatics, resins and asphaltenes on a laboratory scale.[21]

The asphaltene fraction is defined as a solubility class rather than as a chemical class which makes their analysis more difficult. In the past decades there were many uncertainties regarding principles of asphaltene science e.g. molecular and nanocolloidal properties.[25] That was due to inappropriate analytical techniques poorly suited for asphaltenes investigation such as vapor-pressure osmometry (VPO).[17] Recently, asphaltene science has undergone developments owing to the advances in analytical methods. A single fundamental description of asphaltenes has been established and is known as modified Yen model (or Yen-Mullins model).[26] This model states that the predominant molecular architecture of asphaltenes is a single, relatively large polycyclic aromatic hydrocarbon with substituted alkyl chains. It can form nanoaggregates consisting of approximately six individual molecules which can be further combined into clusters. In the following a detailed description of the most important properties is presented. Although, the average elemental composition and molecular weight has been thoroughly investigated and is well-known, there are still uncertainties regarding their chemical structures.

The chemical composition of asphaltenes correlates with their origin.[27] For instance, a typical virgin crude oil asphaltene (UG8) consists of mass percent 81.1 of carbon, 7.1 of hydrogen, 1.0 of nitrogen, 1.6 of oxygen and 8.9 of sulfur.[17] Depending on the source, the average ratio of hydrogen to carbon has a range of 0.4 to 1.2, with the ratio being ca. 1 to 2 in case of the maltene fraction. The presence of significant amounts of heteroatoms (max. 10-15%, *w/w*) is considered to be the cause for the problems during production.[20, 25, 28] Nitrogen-containing compounds are known to be responsible for poisoning of catalysts in chemical processes [25], oxygen species (e.g. naphthenic acids) can lead to corrosion in refineries [29] and the combustion of sulfur-containing compounds is a major cause for acid rain formation [30].

The molecular weight of asphaltenes is one of the crucial parameters to interpret their behavior.[31] After a long period of debates, a consensus was reached based on field ionization mass spectrometry measurements [10] which determines that asphaltenes molecular weight is below 1000 Da (with an average of ca. 750 Da).[16, 17, 26, 32] Contradictory results arise from the fact that asphaltenes can self-associate and they tend to form aggregates in solutions even at low concentrations.[26] The presence of heteroatoms in the aromatic system causes their polar properties and leads to conglomeration and stacking. This might be a possible explanation for the problems on

the production scale. These colloid formations – self-association, flocculation and aggregation – are known to happen especially due to pressure changes and composition changes causing wellbore deposition and fouling.[17, 18]

Determination of exact asphaltene structures is one of the most challenging tasks because it requires investigations on the molecular level. However, general observations can be easily established from the bulk analyte based on certain analytical methods such as ^{13}C NMR or X-ray crystallography.[33] It is suggested that more than half of the carbon atoms are part of an aromatic system (and less than half are aliphatic). Pericondesed (three or more rings share common carbon atoms) aromatic structures are favored over catacondensed (no more than two rings have a common carbon atom) because of the very stable aromatic sextet carbon.[13, 17] An average molecule contains 4-10 six- or five-membered rings, mostly fused (the rings share at least one side) aromatic cores with 6-8 rings, and some rings may be non-aromatic.[12, 34] The diameter of such a molecule is determined to be approximately 10-15 Å (1-1.5 nm).[16, 35] The determination of the construction of the aromatic rings and aliphatic side chains requires fundamental understanding and according to current investigations this is not fully understood. The main question under debate is whether asphaltenes belong to the "island-type" (only one fused aromatic core and the aliphatic chains are overhanging) or the "archipelago-type" (multiple smaller aromatic cores are linked by aliphatic chains) structure is also plausible.[16, 36] Figure 1-3 illustrates some possible structures for a compound with the composition $C_{37}H_{41}N$. The top left molecule consists of one fused ring with several alkyl chains representing an example for an island structure. The other structures are archipelago-type possessing aromatic rings bridged via alkyl chains as well as non-aromatic rings and double bonds in the side chains. The type of the nitrogen-containing compound can be either basic or non-basic (Figure 1-3, bottom left) causing different characteristics for the entire molecule.

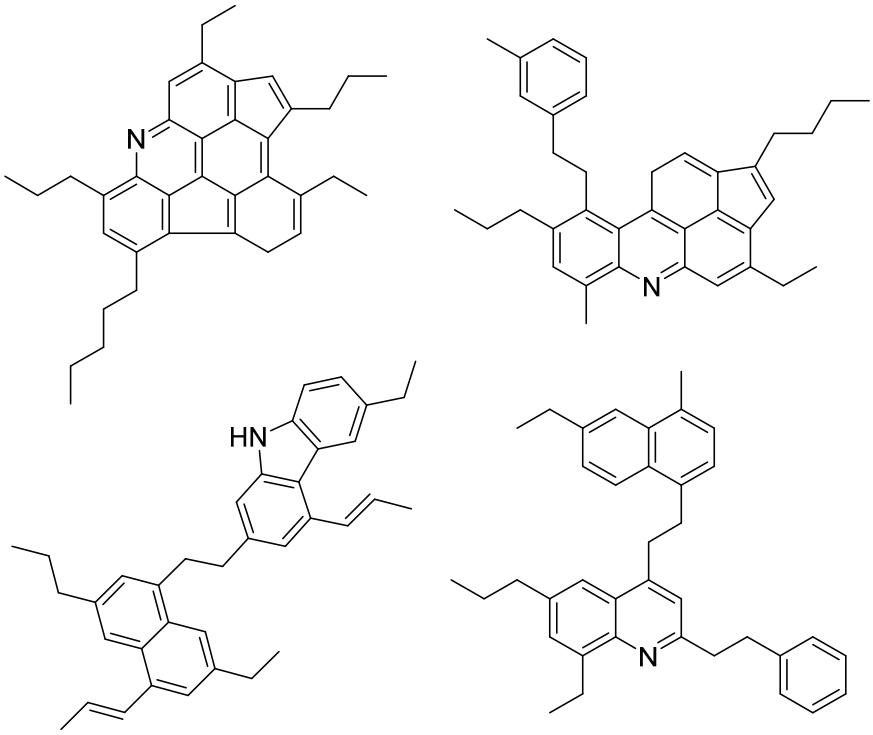

Figure 1-3. Some possible structures for a typical asphaltene of composition $C_{37}H_{41}N$.

1.2 Asphaltene analysis – mass spectrometry

Multiple methods have been used to investigate asphaltenes and other heavier crude oil fractions and to study diverse aspects of these complex samples. These include electron microscopy [37], small-angle neutron and X-ray scattering [38], time-resolved fluorescence depolarization (TRFD) [39], fluorescence correlation spectroscopy (FCS) [40], nuclear magnetic resonance (NMR)[41], vapor-pressure osmometry (VPO) [42] and the two most important methods are mass spectrometry and molecular diffusion to determine molecular weight and size. Since all of these methods demand different criterions and conditions (e.g. high concentrations are needed for VPO measurement, therefore instant gas phase aggregation can lead to wrong interpretation [17]), the established models based on the results can lead to controversies, for example regarding the range of molecular weight, causing debates in the scientific world.[43, 44]

Mass spectrometry is a powerful and sophisticated analytical technique which provides consistent results about a complex mixture such as crude oil consisting of several tens of thousands of different compounds. For the proper characterization of crude oil compounds there are strict requirements with regard to the performance of the analyzers and the features of the ionization technique. It is important to have the

high resolving power to distinguish major isobaric functionalities present in crude oil. For example, to differentiate between C_3 and SH_4 (Δm=3.4 mDa) at around 500 Da mass range a resolving power of >150 000 is required. High mass accuracy is necessary to determine the most probable chemical composition with an error of less than 1 ppm of a given peak. Different analyzers have been developed such as quadrupole, (linear) quadrupole ion trap, time-of-flight mass analyzer, Orbitrap and Fourier transform ion cyclotrone resonance (FT-ICR) cell. Out of all these only the Fourier transform-based techniques (FT-Orbitrap and FT-ICR) can provide sufficiently accurate data, meaning ultrahigh resolution associated with high mass accuracy, to characterize and differentiate individual signals and to be able to correctly assign the elemental compositions present in a complex mixture.[45-47] The analyzers relevant to this research will be discussed in Section 1.2.2. Furthermore, the selection of the ionization source is also an essential question and part of the requirements of the effective crude oil analysis. In order to avoid any undesirable fragmentation, soft ionization methods need to be utilized. Each ionization technique highlights specific types of compound classes. In this regard it has been shown that a combination of different techniques can provide a thorough description in case of such complex mixture.[33]

1.2.1 Ionization techniques

A wide variety of ionization techniques have been introduced to mass spectrometry for different purposes and applications. In crude oil analysis these include (low-voltage) electron ionization (LV)EI [48], chemical ionization (CI) [49], fast atom bombardment (FAB) [50], field desorption/field ionization (FD/FI) [51], laser desorption ionization (LDI) [52], inductively coupled plasma (ICP) [53], and matrix assisted laser desorption ionization (MALDI) [54]. These techniques (except ICP) are applied under vacuum. However, recently for crude oil analysis the most frequently used ionization techniques are carried out under atmospheric pressure, called atmospheric pressure ionization (API). These are electrospray ionization (ESI), atmospheric pressure photoionization (APPI), atmospheric pressure chemical ionization (APCI), atmospheric pressure laser ionization (APLI) [33], and ambient ionization techniques such as desorption electrospray ionization (DESI) [55] and direct analysis in real time (DART).[56] In the followings only the methods used in this research (ESI, APPI and APCI) are described in more detail.

Electrospray ionization

Owing to the diverse polarity of a complex crude oil sample several ionization methods have been developed to be able to study both polar and non-polar compounds. John Fenn presented first that electrospray ionization could easily generate ions from polar species.[57, 58] Briefly, in a positive (or negative) electric field (3-5 kV) applied between the capillary tip and the counter electrode, tiny charged droplets are created from the sample solution which are passing through the capillary needle made of stainless steel. A Taylor cone is formed in this process which releases the smaller charged droplets.[59] Desolvated charged analytes are created either by Coulomb explosion of the droplet after evaporation of the solvent molecules whereby the Raleigh limit of the droplets is reached and the droplet explodes (charged residue model) [60] or by short burst ejection of smaller charged structures (ion evaporation model) [61]. A small portion of these ions enters the orifice of the mass spectrometer at atmospheric pressure through a transfer capillary connected to the first pumping stage of the instrument where the pressure is a few mbar. However, the actual ionization mechanism is still uncertain.[62] Electrospray ionization is a so called soft ionization technique resulting in little or no fragmentation. This is a possible method of choice for the characterization of asphaltenes because it covers the high amount of polar heteroatom-containing compounds which are the most problematic ones in production. One of the main limitations of this technique is that ionization of non-polar molecules is not effective due to their poor ionization ability. However, there are several studies reporting that non-polar compounds possessing more than two condensed rings can be ionized by ESI.[63, 64] This observation was also found during these studies demonstrating that under certain circumstances (proper selection of solvent system and capillary voltage) polyaromatic hydrocarbons and heterocycles can be detected as radical cations.[23] Therefore, it is reasonable that electrospray ionization is one of the essential ionization methods for a full characterization of highly condensed aromatic structures such as asphaltenes.

Atmospheric pressure photoionization

Atmospheric pressure photoionization has become the most important ionization technique for crude oil analysis because it can ionize complex mixtures containing compounds with a broad range of polarity, from non-polar to low-polar and polar

species, simultaneously. The main advantage of APPI over ESI is that the coupling of liquid chromatography to a mass spectrometer is more feasible with this ionization technique. The applied flow rate in case of APPI can be higher therefore more analyte is introduced per unit time which results in higher intensity. In case of APPI a gas discharge lamp (usually krypton lamp) is used as a photon source with a photon emission at 10.0 and 10.6 eV (124 nm and 117 nm, respectively). This emitted energy level needs to be high enough to ionize the organic compounds, but low enough to minimize the ionization of the solvents/air and to avoid fragmentation. Analytes which contain UV chromophores (a region in the molecule possessing π-bonds or lone electron pairs of heteroatoms) are typically ionized by this technique generating mostly both molecular ions and protonated molecules.[65] The sample solution is injected into a heated sprayer, operated at 200-500 ˚C and equipped with a fused silica capillary, where it is instantaneously vaporized and desolvated. Scheme 1-1 shows the pathways of ion formation in positive mode APPI. In case of direct photoionization (1) radical cations are created by absorption of a photon (hν) directly resulting in the ejection of an electron. Alternatively, photons are absorbed by excess surrounding gas-phase solvent molecules (S), creating intermediate radical solvent cations (2). These can lead to the ionization of the analyte molecules (M) by subsequent charge exchange (3). An easily ionisable reagent, typically toluene or acetone, can serve as a dopant (D) to directly help the ionization of the analytes by charge exchange (4) or through proton transfer (5).[66] Therefore, toluene is an excellent choice as solvent for aspheltene analysis because it simultaneously acts as a dopant, resulting in effective ionization and increased sensitivity.

$$(1)\quad M + h\nu \rightarrow M^{\bullet+} + e^-$$

$$(2)\quad S + h\nu \rightarrow S^{\bullet+} + e^-$$

$$(3)\quad S^{\bullet+} + M \rightarrow S + M^{\bullet+}$$

$$(4)\quad D^{\bullet+} + M \rightarrow D + M^{\bullet+}$$

$$(5)\quad D^{\bullet+} + M \rightarrow [D-H]^{\bullet} + [M+H]^{+}$$

Scheme 1-1. Reaction cascade for ion formation in positive mode APPI.

Atmospheric pressure chemical ionization

The same heated sprayer, which was already described for the APPI, is used in atmospheric pressure chemical ionization, where the ionization is carried out using a corona discharge needle set to a high potential (2-5 kV) which is placed near the orifice of the MS. The resulting electrical discharge (corona discharge) leads to a plasma region, where the actual analyte ionization takes place. First, the ambient nitrogen is ionized, which then leads to the subsequent ionization of the analyte compounds through ion-molecule reactions, proton transfer and/or adduct formation in the gaseous phase.[67, 68] As a result of molecular collisions and charge transfer, ionized gas plasma is produced in the corona discharge region. The reactions in this plasma region are shown in Scheme 1-2. Primary $N_2^{\bullet+}$ ions (1) collide with atmospheric water vapour present in the source (2a) to create secondary reactant gas ions, $[H_2O]_{n+1}H^+$ cluster ions (2b,c,d). These reactant gas ions then undergo collisions with the analyte (M) (3) to result in analyte ions by proton transfer.

(1) $e^- + N_2 \rightarrow N_2^{\bullet+} + 2e^-$

(2a) $N_2^{\bullet+} + H_2O \rightarrow N_2 + H_2O^{\bullet+}$

(2b) $H_2O^{\bullet+} + H_2O \rightarrow H_3O^+ + HO^{\bullet}$

(2c) $H_3O^+ + H_2O + N_2 \rightarrow [H_2O]_2H^+ + N_2$

(2d) $[H_2O]_nH^+ + H_2O + N_2 \rightarrow [H_2O]_{n+1}H^+ + N_2$

(3) $H_3O^+ + M \rightarrow MH^+ + H_2O$

Scheme 1-2. Gas-phase reaction scheme for positive mode APCI.

1.2.2 Mass analyzers

In order to achieve high resolving power and high mass accuracy, techniques with powerful mass analyzers based on Fourier transform such as FT-ICR and FT-Orbitrap need to be used which can fulfil the necessary requirements for complex mixture analysis. In both cases, the charged species are forced to make periodic circulating motions (cyclotron motion or harmonic oscillation) by an electromagnetic or electrostatic field. All circulating ions with the same *mass-to-charge* ratio oscillate at the same frequency, independent of their kinetic energy. This movement induces an image current which is detected as a time-dependent signal. This decaying time domain signal,

called transient, is then converted into the frequency domain signal by Fourier transform. The ability to accurately measure time and consequently the ions' frequencies makes it possible to achieve high mass resolution. Unlike the other detectors such as electron multiplier where the detection is based on collisions, these are non-destructive, so ions can be excited and relaxed multiple times. By accumulating multiple subsequent individual scans (meaning enhanced measurement time), an enhanced *signal-to-noise* ratio can be achieved resulting in an increased resolving power.

Fourier transform ion cyclotron resonance mass spectrometry

FT-ICR was developed as a combination of Fourier transform and ion cyclotron resonance to make use of the principle for mass spectrometry by Marshall et al. in 1974.[69, 70] A strong magnetic field provided by a superconducting magnet forces the ions trapped in the ICR cell onto a cyclotron motion. Commercial instruments are currently available with magnet field strength of 7, 9.4, 12 and 14.5 Tesla. A 21 Tesla magnet has been installed recently which is the strongest magnet ever used for FT-ICR featuring high spatial homogeneity, high temporal stability and low liquid helium consumption.[71]

The commercial cyclotron cell is cylindrical but other cell designs are also available.[70] Optimization of the cylindrical cell is a question of interest to make the best use of the cell with better field distribution therefore aiming for less ion-ion interaction.[72] It consists of three opposing pairs of electrode plates serving the three main important functions such as trapping, excitation and detection. Figure 1-4 illustrates the schematic diagram of the cell. After trapping externally generated ions, coherent ion packets are formed which need to be excited to move into a larger cyclotron orbit to get closer to the detection plates. Excitation is carried out by an RF pulse or RF sweep covering a broader frequency range and the ions gain extra energy when the RF field equals to their cyclotron frequency.[70] This energy excess leads them to move into a larger orbit. As the ions are excited, detection of the much stronger electrical signal (mirror current) can take place after amplification. With time during the detection period, the ions lose their energy resulting in a weakening transient signal. The longer the ions circulation can be detected the more precise the measurement is therefore the higher the resolving power becomes. Each ion has a certain frequency in a given magnetic field which produces distinct signals. The transient time-dependent

signal is converted into frequency domain spectrum by Fourier transform which gives then the well-known mass spectrum: ion abundance as the function of *mass-to-charge* (*m/z*) ratio.

A 7 Tesla FT-ICR MS is used for some experiments in this study which allows a maximum resolving power of 1 000 000 (FWHM at *m/z* 400) with a transient time of approximately 12.5 seconds.

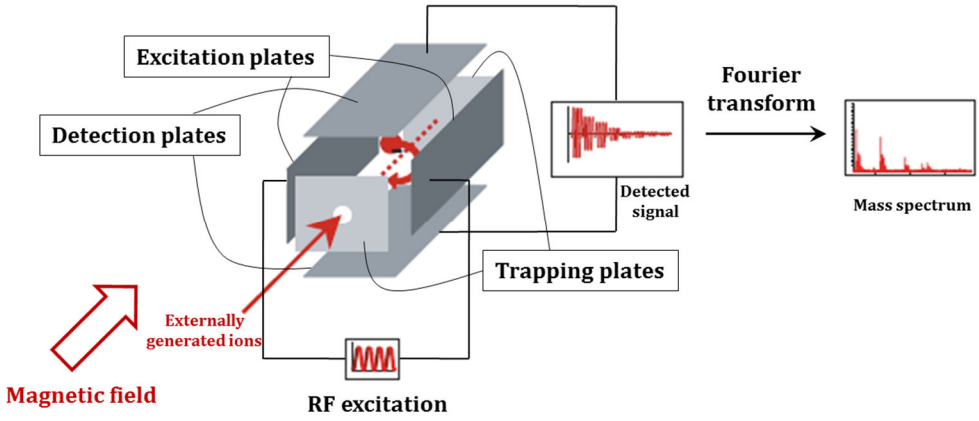

Figure 1-4. Schematic diagram of the cyclotron cell consisting of two trapping plates, two excitation plates and two detection plates adapted from Schrader et al.[73]

Orbitrap

The Orbitrap analyzer was invented by Makarov et al. and was introduced commercially in 2005.[74, 75] It consists of two coaxial electrode sets, a spindle-shaped central electrode on the axis and a hyperconical split electrode on the outside between which there is a defined volume where the ions are trapped. A high voltage is applied to the central electrode resulting in an inhomogeneous electrostatic field throughout the Orbitrap. On the one hand ions orbit around the central inner electrode on elliptical trajectories balanced by the centrifugal forces and on the other hand they move back and forth along the axis. The axial motion of the trapped ions is a harmonic oscillation, which is dependent only on the ions *mass-to-charge* ratios and independent from the ions motion around the inner electrode and from other properties.[74] There is no need for any additional excitation; the image current of this axial oscillation of ion rings is detected directly on the outer electrode over a certain period of time. Higher resolving power can be achieved by increasing the detection time or the strength of the field.

A curved linear trap, called C-trap, is used to execute the injection from an external ion source. As ions are combined into packets and are injected tangentially to the central axis of the trap, an increasing voltage (voltage ramp) is applied to squeeze the ions towards the central electrode. Once the ions gain the desired trajectories, the electric field becomes static, as well. The different ions having different *mass-to-charge* ratio move with different frequencies creating separate rotational rings along the central axis. A schematic illustration can be seen in Figure 1-5.

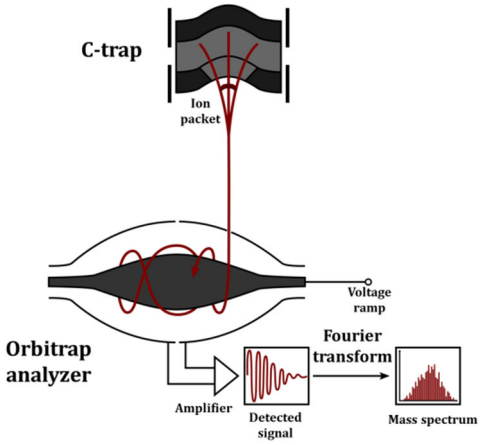

Figure 1-5. Schematic illustration of the Orbitrap analyzer equipped with C-trap.

There are two variants of Orbitrap: the standard Orbitrap and the high-field Orbitrap. The shape of the outer electrodes is identical in both cases; however, in the new design (high-field) the gap between the inner and outer electrodes is decreased, thus reaching higher field strength for a fixed voltage. Higher field strength results in higher frequencies of the oscillating ions thereby over a given acquisition time leading to higher resolving power.[76] A new research-type high-field Orbitrap is used in this study to characterize complex mixtures. This allows a maximum resolving power of 960 000 (FWHM at *m/z* 400) with a transient time of 3 seconds.

Linear quadrupole ion trap

The linear quadrupole ion trap has been commercially incorporated in many so called hybrid mass spectrometers such as FT-ICR [77] and FT-Orbitrap [78] instruments. These types of hybrid mass spectrometers were used during these studies making profit of an enhanced performance.

The linear quadrupole ion trap is similar to the widely used quadrupole ion trap or Paul trap [79-81], but instead of a three-dimensional quadrupolar field, the ions are

trapped in a two-dimensional field [82]. While the quadrupole ion trap consists of one ring electrode and two endcaps (top and bottom) and ions coming through one of the caps are oscillating in a 3D space, in a linear ion trap hyperbolic quadrupole rods and end electrodes (front and back section) are used to trap the ions (Figure 1-6). In order to remove the ions excess energy, a low helium flow is applied as cooling gas so that the ions in the trap stay stable and oscillate in the *xy* plane line in the center section. By manipulation of the electrode RF potentials, the ions are getting unstable according to their *m/z* ratios and are ejected towards the detector which might be either the electron multipliers (two multipliers on the sides) or one of the FT-based analyzer cells (by removing the trapping potential from the back section).

Compared to a quadrupole ion trap, the linear ion trap has an increased ion storage capacity and enhanced trapping efficiency for externally injected ions enabling reduced space charge effects and higher sensitivity, respectively.[77, 82, 83] Using an ion trap as preselection element prior to a high resolving FT MS analysis allows for the application of automated gain control (AGC) to control the number of ions transferred to the analyzer.[84] This minimizes space charge dependent mass shifts and therefore enables high accuracy mass measurements.[84] AGC control becomes especially important when running analyses is accomplished with varying analyte amounts, such as during LC/MS couplings. The combination of a linear ion trap and an FT-based mass analyzer into a hybrid instrument therefore enables precise mass measurements along with MS^n-capabilities.[77]

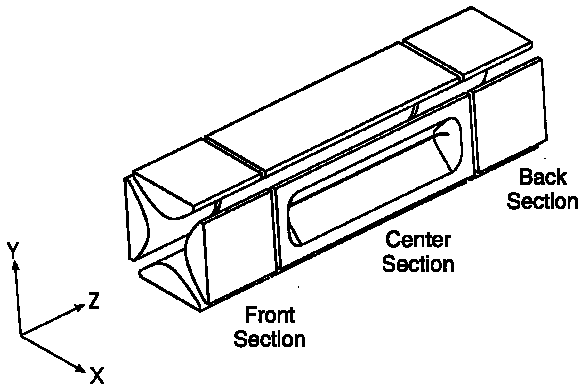

Figure 1-6. Schematic design of two-dimensional linear ion trap.[82]

1.2.3 Hybrid FT mass spectrometer

In Figure 1-7 the schematic diagram illustrates the hybrid FT mass spectrometers used in this study showing the whole setup with the most important parts (API ion source, linear ion trap, and FT-based mass analyzer with ion guides) with the operating pressures.

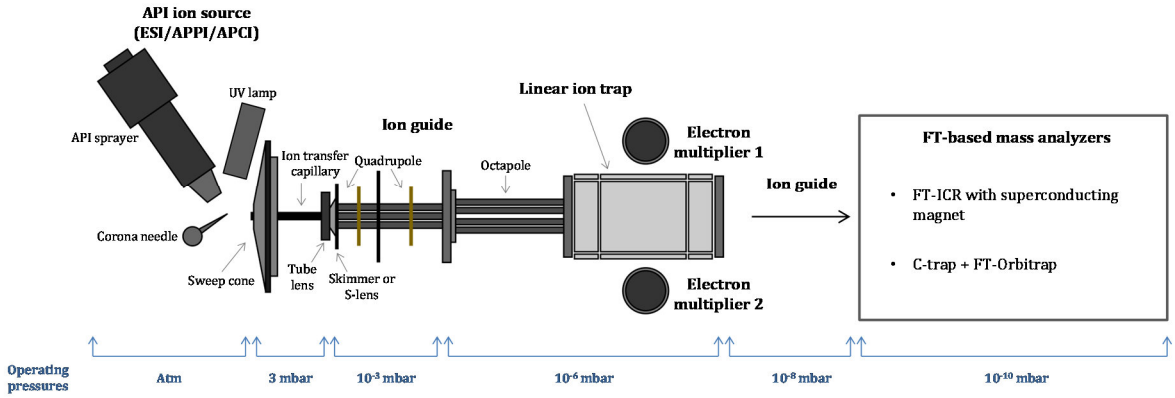

Figure 1-7. Schematic diagram of hybrid mass spectrometer with the operating pressures.

1.3 Tandem mass spectrometry

Tandem mass spectrometry, also known as MS/MS or MS^n, incorporates fragmentation of selected ions at multiple stages. Tandem mass spectrometry measurements can be accomplished either in space or in time.[85] Tandem-in-space means that the elements participating in the fragmentation experiment such as quadrupoles are physically separated and connected successively working either as mass analyzer or collision chamber.[86] In the case of tandem-in-time, the ions are trapped in a defined place and all the individual steps, such as separation, ion manipulation and fragmentation, are occurring here consecutively in time. This procedure can be repeated multiple times in the latter case (MS^n).[81] Therefore, deeper structural information can be achieved owing to multiple steps of fragmentation. As it was mentioned before, the linear ion trap is capable of performing such an analysis making it a very powerful analytical tool (see page 14).

In this study collision-induced dissociation (CID) was applied for MS/MS fragmentation experiments. CID is the most common activation technique where

precursor ions are isolated then excited by increasing their kinetic energy via application of RF potential.[87, 88] These ions with increased kinetic energy undergo (single or multiple) collisions with a neutral atom or molecule in the gas phase. Helium (in hybrid instruments) and argon (triple quadrupole) are typically used as buffer gases. Consequently, the kinetic energy is converted into internal energy resulting in subsequent dissociation into fragment ions.[88, 89]

The combination of the linear ion trap and FT-based instruments makes it possible to achieve high-resolution data of the fragmentation measurements. All the ion manipulations (isolation, activation and fragmentation) occur in the linear ion trap, from where the targeted ions are ejected and transferred through lenses and multipoles into the FT-ICR or the C-trap of FT-Orbitrap for detection.

1.4 Complexity reduction of heavy crude oil

As it was pointed out previously, mass spectrometry is a powerful tool in crude oil analysis which can provide information about elemental compositions based on the detection of *mass-to-charge* ratios of ionized species; however, it has certain limitations if the analyte is extremely complex. For example, space charge effects originating from the impact of electric fields of the trapped ion packets in the analyzer cell might lead to mass shifts and improper detection.[90] Furthermore, it is known that matrix effects, ion suppression, ion discrimination effects and instrument sensitivity are playing a significant role in limiting the number and the type of detected species.[33, 91-93] All of these effects can be reduced or eliminated if a lower amount of different species is present in the cell during each transient whereby less ion-ion interaction can take place. This can be achieved by simplification of the sample. Although, SARA fractionation is a standard separation step in heavy crude oil analysis, the complexity of the resulted fractions is still considerably high and therefore further simplification is needed. Application of chromatographic separation is often the method of choice for complexity reduction and simplification. Depending on the chosen method, separation can be accomplished based on certain selected properties (e.g. polarity, aromaticity, molecular size).

In conclusion, a comprehensive picture with increased information depth about the whole crude oil sample can only be achieved if prior separation methods are applied.

1.5 Data interpretation in crude oil analysis

Mass spectrometry is a powerful tool to analyze complex crude oil samples. The aim is to identify and characterize all of the components which are present in the complex system. It is a non-target analysis where information is required about all of the obtained signals; therefore the question of interest does not narrow down to specific components or compound groups. In order to get as many precise assignments as possible, high resolving power and high mass accuracy of the mass spectrometer is essential.

A mass spectrum of a crude oil sample consists of tens of thousands peaks. In order to correlate them with the correct chemical compositions, the signals have to be assigned based on certain constrains and preliminary expectations. For example, the components contain carbon, hydrogen, nitrogen, oxygen and sulfur as major elements. For validation of the possible elemental compositions, isotopic fingerprints have to be used to confirm the data. Since crude oil contains a large amount of hydrocarbons with a high number of carbon atoms, ^{13}C isotopic patterns are mostly used for this verification purpose. Statistically, 1.1% of all of the carbon atoms are present as ^{13}C isotope (and 98.9% as ^{12}C), therefore the peak corresponding to the molecular formula including one ^{13}C has to be present in the mass spectrum, as well. This should be observed exactly 1.00335 Da higher than the signal of the monoisotopic species (assuming exclusively singly charged species in crude oil [45]).

Since there are no specific target molecules in crude oil analysis, instead of individual peaks, homologous series are calculated. It is because crude oil is known to consist of series of compounds with the same aromatic core and heteroatom content but with varying length of their aliphatic carbon chains. Figure 1-8 (top and middle) shows the full mass spectrum and mass scale-expanded views where the repeating units of CH_2 with a mass difference of 14.01565 Da are marked with stars. The assignments and molecular formulae corresponding to these peaks belong to the same heteroatom class having the same unsaturation (same hydrogen deficiency) but different carbon number, therefore different alkylation state. Additionally, in Figure 1-8 (bottom) a mass difference of 2.01565 Da can be recognized which corresponds to the difference of H_2. The compounds corresponding to these series of peaks have a different unsaturation

state (different hydrogen deficiency) with the same carbon content, which is associated with their aromaticity.[94]

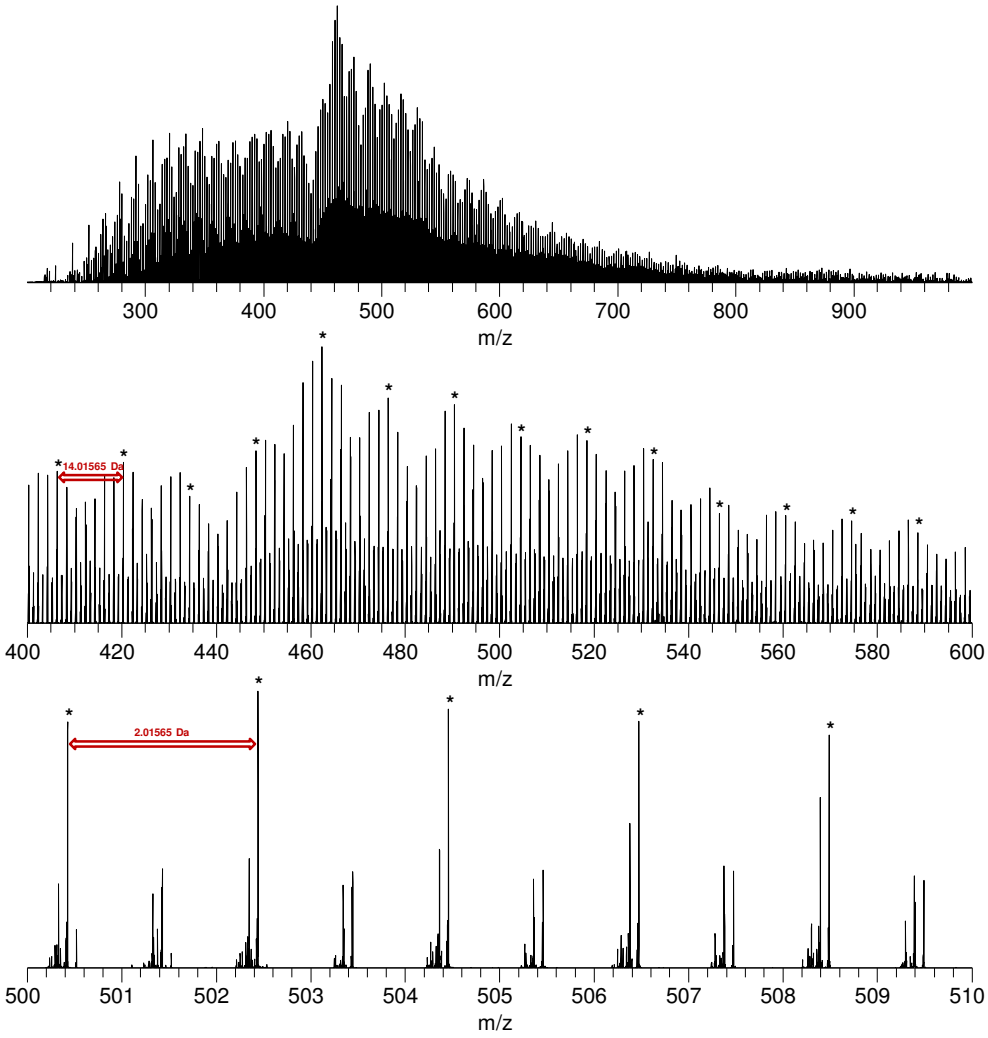

Figure 1-8. ESI mass spectrum of a crude oil sample (top) and mass scale-expanded views in the mass range of 400-600 *m/z* (middle) and 500-510 *m/z* (bottom).

Since in a crude oil sample the importance of homologous series is emphasized, using CH_2 as a unit proved beneficial and it is often utilized in representations. In order to simplify the representation, observed masses (with a IUPAC unit of m(^{12}C) = 12.00000 Da, therefore m($^{12}CH_2$) = 14.01565 Da) are converted to the so called Kendrick mass scale.[95] In Kendrick mass scale the basic unit is CH_2 (m($^{12}CH_2$) = 14.00000 Da). Consequently, the Kendrick mass (KM) can be calculated according to the equation:

$$KM = observed\ mass \times \frac{14.00000}{14.01565}$$

Additionally, the Kendrick nominal mass (KNM) is interpreted as the nearest integer and the Kendrick mass defect (KMD) is defined as the difference according to:

$$KMD = (KNM - KM) \times 1000$$

After all of these calculations, the full representation of a complex crude oil sample can be revealed by a two-dimensional graph where KMD is plotted against KNM which is shown in Figure 1-9. Here each point depicts one peak from the mass spectrum. In the different horizontal rows, meaning different mass defects, heteroatom classes are distinguished with different colors. Furthermore, different rows with the same color mean different hydrogen deficiencies for the same heteroatom class. Within each horizontal row the interval of 14 Da represents compounds which differ in the amount of CH_2 units and therefore in alkylation state.

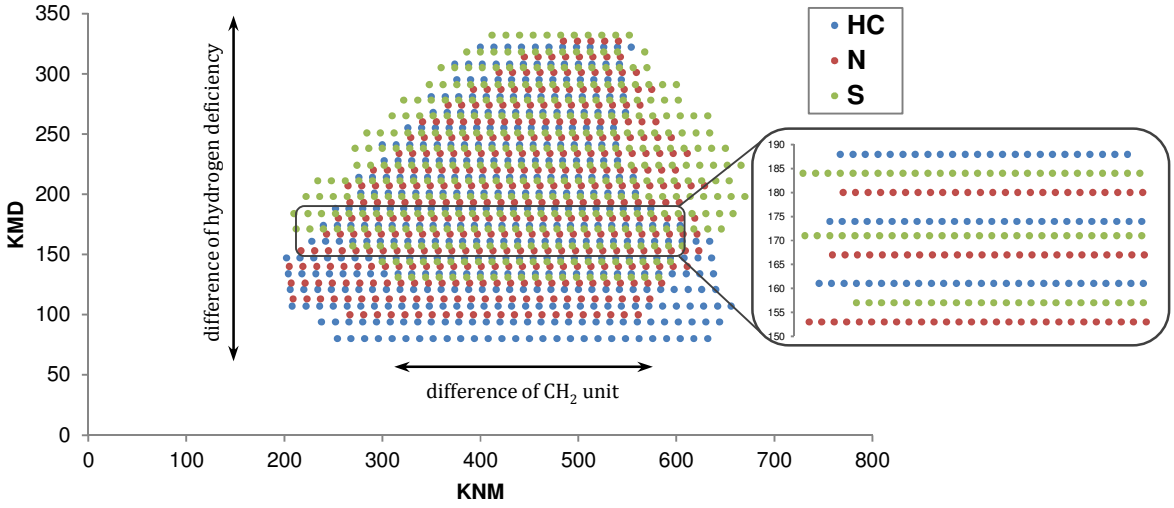

Figure 1-9. Plot of Kendrick mass defect (KMD) vs. Kendrick nominal mass (KNM) of a crude oil sample.

Aromaticity of a crude oil sample is always a question of interest and there are several possibilities to reflect it. One of them is double bond equivalent (DBE) which represents the sum of the number of rings and double bonds within a molecule. This is calculated from the known elemental formula of $C_cH_hN_nO_oS_s$ according to the equation:

$$DBE = c - \frac{h}{2} + \frac{n}{2} + 1$$

Each additional double bond or aromatic ring increases the DBE value. The DBE versus carbon number plot (Figure 1-10, A) is often used for compounds containing hydrocarbons and polyaromatic heterocycles to visually represent the increased number of aromatic rings. A normalization of DBE value to the number of carbon atoms (DBE/C) is another way to illustrate the degree of aromaticity (Figure 1-10, B) which

helps to identify species with condensed aromatic ring structures (CARS), that fall between the DBE/C values of 0.7 and 0.9 [96].

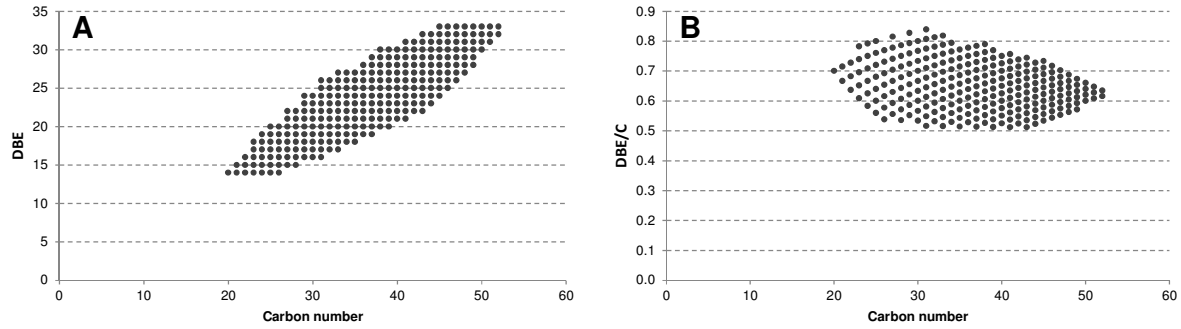

Figure 1-10. Examples for practical representation of heavy crude oil data.
A. DBE versus carbon number B. DBE/C versus carbon number.

Another aspect which provides information about the aromaticity of a complex system is the plot of the molar ratio of hydrogen-to-carbon (H/C ratio) versus the molar ratio of "heteroatom-to-carbon" (N/C, O/C or S/C ratio) of the assigned elemental formulae which is known as van Krevelen diagram.[97] This approach is often used to represent different chemical reactions in which loss or gain of elements in a certain molar ratio is involved (e.g. methylation, oxidation, decarboxylation) where the trend lines for the specific reactions can be clearly distinguished.[98] H/C ratio ranges from 2 to 0.4; the lower the value, the more aromatic the system is. As long as the overall distribution of compounds with all of the heteroatoms is in question, Kendrick plot is recommended. However, if the focus is on a certain heteroatom, especially the comparison of the different numbers of a selected heteroatom, then a van Krevelen diagram is advisable. Figure 1-11 shows an example where N_1, N_2 and N_3 classes are displayed and compared in case of the asphaltene fraction of crude oil. Here the different regions containing different numbers of nitrogen atoms can be clearly discerned.

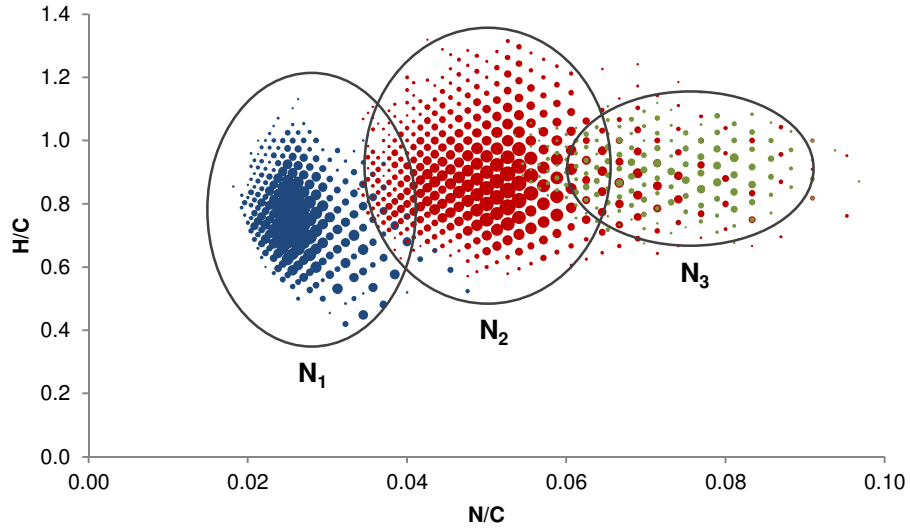

Figure 1-11. van Krevelen diagram of the N_1, N_2 and N_3 classes of an asphaltene fraction originated from a heavy crude oil sample observed in positive ESI (dot size is proportional to the relative intensity).

Further visualization of the data includes heatmaps where besides the information of e.g. DBE at different masses or carbon numbers, the intensities or relative abundance of the assigned peaks are also included and color-coded as the z-axis (Figure 1-12, A). For statistical representation of the data, Box plots are often used where information such as smallest and largest observation data, lower and upper quartile (25% and 75%) and mean value can be displayed (Figure 1-12, B).

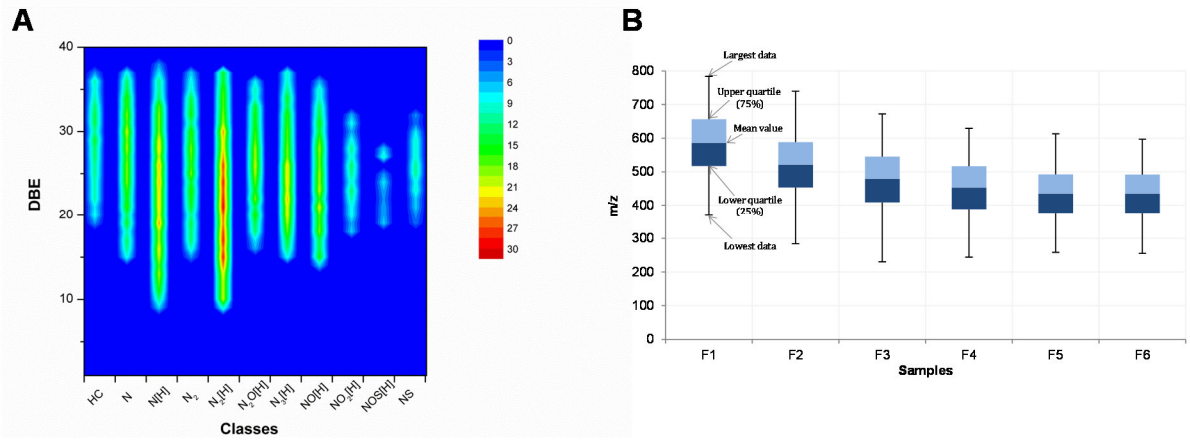

Figure 1-12. Visualization of crude oil data. A. Heatmap of DBE versus classes (population is color-coded) B. Box plot of *m/z* distribution of different samples.

These latter representations which include more than two types of information, depending on the target, can facilitate the visualization of complex samples by avoiding the complications of multiple plots.

1.6 Aim of the study

The complexity of heavy crude oil and crude oil asphaltenes is a central issue. Mass spectrometry with different ionization techniques is a feasible analytical tool for investigation of such a complex mixture possessing compounds with diverse polarity. Application of tandem mass spectrometry measurements enables also the characterization of molecular structures of desired compounds. Prior chromatographic separation simplifies the sample based on certain properties and eliminates matrix effects. The scope of this study was the development of analytical methods for a comprehensive characterization of individual compounds in very complex crude oil mixtures. The main objective was that these analytical methods should allow gaining structural information of individual chemical components. In order to achieve these goals, different techniques needed to be developed.

In Chapter 2 it is presented that although electrospray ionization is usually used for polar compounds, under controlled conditions it is capable to ionize non-polar hydrocarbons, as well. Polyaromatic hydrocarbons and heterocycles can form radical cations in the ESI source which operates as an electrolytic flow cell facilitating electrochemical oxidation of the condensed aromatic structures. As a consequence, the usability of ESI can be extended and highly aromatic non-polar hydrocarbons can be successfully analyzed, as well.

Chapter 3 demonstrates the successful application of size exclusion chromatography for asphaltene compounds and its direct coupling to an ultrahigh resolving mass spectrometer. This work is based on previous studies from our group, were the conditions were optimized, and furthermore the previously used harmful solvent (benzene) has been replaced. The separation results only in one although fairly broad peak with a time width of 6 min and the compounds are separated according to their size. Bigger compounds are eluting first and the smaller ones later depending on the different exclusion from the pores of the stationary phase. The fast online coupling makes it possible to collect all fractions from the separation with different time windows.

Another chromatographic separation method, namely argentation chromatography has been investigated in Chapter 4. The operating conditions has been optimized for heavy crude oil containing compounds with high amount of heteroatoms (N, O, S) and

high degree of unsaturation. Separation by argentation chromatography can be achieved based on weak and reversible interactions between the silver ions (Ag^+) immobilized on the silica gel surface and the π-bonds and/or heteroatoms of unsaturated aromatic compounds. It is demonstrated that using silver-modified silica gel with 5 μm particle size as stationary phase can highlight compounds within a specifically high DBE range (30-35).

The application of argentation chromatography has been extended to the pure asphaltene compounds. This new separation approach for asphaltenes is introduced in Chapter 5. Here the difficulty arose from their highly condensed aromatic feature. These kinds of compounds tend to create very strong interaction with the stationary phase. This created the need for developing another solution that allows the separation of such highly aromatic compounds using a much stronger competitive solvent, dimethyl sulfoxide instead of the previously used isopropanol. The efficiency of separation of asphaltenes is demonstrated here by using argentation chromatography online coupled with ultrahigh-resolution mass spectrometry.

Application of consecutive separation methods (size exclusion chromatography and argentation chromatography) together with fraction collection makes it possible to simplify the extreme complex mixture by reducing the amount of different species at the same time. Chapter 6 is focusing on the investigation of the effectiveness of combination of chromatographic separations and the study of resulting individual compounds.

Structural elucidation of heavy crude oil fractions (asphaltenes and maltenes) is a challenging task which was the final objective of this thesis. The separation procedure embraced with tandem mass spectrometric measurements with high resolution and high mass accuracy provides the possibility to perform structural determination of individual chemical compounds of such complex samples which is demonstrated in Chapter 7. In the end, the analytical methods developed here make it possible to describe the differences in the chemical structures between asphaltenes and maltenes.

Finally, the overall conclusion of this study is presented in Chapter 8.

1.7 References for Chapter 1

[1] O. Ellabban, H. Abu-Rub, F. Blaabjerg. Renewable energy resources: Current status, future prospects and their enabling technology. *Renew. Sust. Energ. Rev.* **2014**, *39*, 748-764.

[2] S. H. Mohr, G. M. Evans. Long term prediction of unconventional oil production. *Energy Policy* **2010**, *38*, 265-276.

[3] W. Youngquist. Shale oil - the exclusive energy. *Hubbert Center Newsletter* **1998**, *98/4*.

[4] E. C. Sanford. Processibility of athabasca oil sand: Interrelationship between oil sand fine solids, process aids, mechanical energy and oil sand age after mining. *Can. J. Chem. Eng.* **1983**, *61*, 554-567.

[5] J. Czarnecki, B. Radoev, L. L. Schramm, R. Slavchev. On the nature of Athabasca Oil Sands. *Adv. Colloid Interface Sci.* **2005**, *114–115*, 53-60.

[6] Petroleum Services Association of Canada.

[7] S. Sorrell, R. Miller, R. Bentley, J. Speirs. Oil futures: A comparison of global supply forecasts. *Energy Policy* **2010**, *38*, 4990-5003.

[8] M. M. Boduszynski. Composition of heavy petroleums. 1. Molecular weight, hydrogen deficiency, and heteroatom concentration as a function of atmospheric equivalent boiling point up to 1400.degree.F (760.degree.C). *Energy Fuels* **1987**, *1*, 2-11.

[9] J. Speight. Handbook of Petroleum Analysis, Wiley Interscience, New York, **2001**.

[10] M. M. Boduszynski. Composition of Heavy Petroleums. 2. Molecular Characterization. *Energy Fuels* **1988**, *2*, 597–613.

[11] A. M. McKenna, J. M. Purcell, R. P. Rodgers, A. G. Marshall. Heavy Petroleum Composition. 1. Exhaustive Compositional Analysis of Athabasca Bitumen HVGO Distillates by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: A Definitive Test of the Boduszynski Model. *Energy Fuels* **2010**, *24*, 2929-2938.

[12] A. M. McKenna, G. T. Blakney, F. Xian, P. B. Glaser, R. P. Rodgers, A. G. Marshall. Heavy Petroleum Composition. 2. Progression of the Boduszynski Model to the Limit of Distillation by Ultrahigh-Resolution FT-ICR Mass Spectrometry. *Energy Fuels* **2010**, *24*, 2939-2946.

[13] K. Akbarzadeh, A. Hammami, A. Kharrat, D. Zhang, S. Allenson, J. Creek, S. Kabir, A. Jamaluddin, A. G. Marshall, R. P. Rodgers, O. C. Mullins, T. Solbakken. Asphaltenes—Problematic but Rich in Potential. *Oilfield Rew.* **2007**, *19*, 22-43.

[14] A. Hammami, J. Ratulowski, in *Asphaltenes, Heavy Oils, and Petroleomics* (Eds.: O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall), Springer New York, **2007**, pp. 617-660.

[15] S. Chopra, L. Lines. Special section: Heavy Oil. *The Leading Edge* **2008**, *27*.

[16] O. C. Mullins. The Asphaltenes. *Annu. Rev. Anal. Chem.* **2011**, *4*, 393-418.

[17] O. C. Mullins. Review of the Molecular Structure and Aggregation of Asphaltenes and Petroleomics. *SPE-95801-PA* **2008**, *13*, 48-57.

[18] R. B. de Boer, K. Leerlooyer, M. R. P. Eigner, A. R. D. van Bergen. Screening of Crude Oils for Asphalt Precipitation: Theory, Practice, and the Selection of Inhibitors. *SPE-24987-PA* **1995**, *10*, 55-61.

[19] T. M. C. Pereira, G. Vanini, L. V. Tose, F. M. R. Cardoso, F. P. Fleming, P. T. V. Rosa, C. J. Thompson, E. V. R. Castro, B. G. Vaz, W. Romão. FT-ICR MS analysis of asphaltenes: Asphaltenes go in, fullerenes come out. *Fuel* **2014**, *131*, 49-58.

[20] K. H. Altgelt, M. M. Boduszynski. Composition and analysis of heavy petroleum fractions. *Vol. 54*, CRC Press, **1994**.
[21] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Characterization of Saturates, Aromatics, Resins, and Asphaltenes Heavy Crude Oil Fractions by Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2012**, *26*, 3481-3487.
[22] Y. Cho, J.-G. Na, N.-S. Nho, S. Kim, S. Kim. Application of Saturates, Aromatics, Resins, and Asphaltenes Crude Oil Fractionation for Detailed Chemical Characterization of Heavy Crude Oils by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Equipped with Atmospheric Pressure Photoionization. *Energy Fuels* **2012**, *26*, 2558-2565.
[23] L. M. Guricza, W. Schrader. Electrospray ionization for determination of non-polar polyaromatic hydrocarbons and polyaromatic heterocycles in heavy crude oil asphaltenes. *J. Mass Spectrom.* **2015**, *50*, 549-557.
[24] H. Alboudwarej, J. Beck, W. Y. Svrcek, H. W. Yarranton, K. Akbarzadeh. Sensitivity of Asphaltene Properties to Separation Techniques. *Energy Fuels* **2002**, *16*, 462-469.
[25] O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall. Asphaltenes, Heavy Oils, and Petroleomics, Springer, New York, **2007**.
[26] O. C. Mullins. The Modified Yen Model. *Energy Fuels* **2010**, *24*, 2179-2207.
[27] G. C. Klein, S. Kim, R. P. Rodgers, A. G. Marshall. Mass Spectral Analysis of Asphaltenes. I. Compositional Differences between Pressure-Drop and Solvent-Drop Asphaltenes Determined by Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2006**, *20*, 1965–1972.
[28] J. Speight. The Chemistry and Technology of Petroleum, Fifth Edition ed., Taylor & Francis Group, New York, **2014**.
[29] C. S. Hsu, G. J. Dechert, W. K. Robbins, E. K. Fukuda. Naphthenic Acids in Crude Oils Characterized by Mass Spectrometry. *Energy Fuels* **1999**, *14*, 217-223.
[30] J. K. Nduka, O. E. Orisakwe. Precipitation chemistry and occurrence of acid rain over the oil-producing Niger Delta region of Nigeria. *Scientific World J.* **2010**, *10*, 528-534.
[31] E. Y. Sheu. Petroleum AsphalteneProperties, Characterization, and Issues. *Energy Fuels* **2002**, *16*, 74-82.
[32] R. Rodgers, A. Marshall, in *Asphaltenes, Heavy Oils, and Petroleomics* (Eds.: O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall), Springer New York, **2007**, pp. 63-93.
[33] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Impact of Different Ionization Methods on the Molecular Assignments of Asphaltenes by FT-ICR Mass Spectrometry. *Anal. Chem.* **2012**, *84*, 5257-5267.
[34] H. Groenzin, O. C. Mullins. Molecular Size and Structure of Asphaltenes from Various Sources. *Energy Fuels* **2000**, *14*, 677-684.
[35] G. W. Zajac, N. K. Sethi, J. T. Joseph, D. J. Thomson, P. S. Weiss. Molecular imaging of petroleum asphaltenes by scanning tunneling microscopy: verification of structure from 13C and proton NMR data. *Scanning Microscopy* **1994**, *8*, 463-470.
[36] O. P. Strausz, T. W. Mojelsky, E. M. Lown. The molecular structure of asphaltene: an unfolding story. *Fuel* **1992**, *71*, 1355-1363.
[37] A. Sharma, H. Groenzin, A. Tomita, O. C. Mullins. Probing Order in Asphaltenes and Aromatic Ring Systems by HRTEM. *Energy Fuels* **2002**, *16*, 490-496.

[38] J. Eyssautier, P. Levitz, D. Espinat, J. Jestin, J. Gummel, I. Grillo, L. Barré. Insight into Asphaltene Nanoaggregate Structure Inferred by Small Angle Neutron and X-ray Scattering. *J. Phys. Chem. B* **2011**, *115*, 6827-6837.

[39] H. Groenzin, O. C. Mullins, in *Asphaltenes, Heavy Oils, and Petroleomics* (Eds.: O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall), Springer New York, New York, NY, **2007**, pp. 17-62.

[40] A. B. Andrews, R. E. Guerra, O. C. Mullins, P. N. Sen. Diffusivity of Asphaltene Molecules by Fluorescence Correlation Spectroscopy. *J. Phys. Chem. A* **2006**, *110*, 8093-8097.

[41] J. Tong, X. Han, S. Wang, X. Jiang. Evaluation of Structural Characteristics of Huadian Oil Shale Kerogen Using Direct Techniques (Solid-State 13C NMR, XPS, FT-IR, and XRD). *Energy Fuels* **2011**, *25*, 4006-4013.

[42] H. W. Yarranton, H. Alboudwarej, R. Jakher. Investigation of Asphaltene Association with Vapor Pressure Osmometry and Interfacial Tension Measurements. *Ind. Eng. Chem. Res.* **2000**, *39*, 2916-2924.

[43] A. A. Herod, K. D. Bartle, R. Kandiyoti. Characterization of Heavy Hydrocarbons by Chromatographic and Mass Spectrometric Methods: An Overview. *Energy Fuels* **2007**, *21*, 2176-2203.

[44] O. C. Mullins, B. Martínez-Haya, A. G. Marshall. Contrasting Perspective on Asphaltene Molecular Weight. This Comment vs the Overview of A. A. Herod, K. D. Bartle, and R. Kandiyoti. *Energy Fuels* **2008**, *22*, 1765-1773.

[45] C. A. Hughey, R. P. Rodgers, A. G. Marshall. Resolution of 11 000 Compositionally Distinct Components in a Single Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrum of Crude Oil. *Anal. Chem.* **2002**, *74*, 4145-4149.

[46] A. G. Marshall, R. P. Rodgers. Petroleomics: The Next Grand Challenge for Chemical Analysis. *Acc. Chem. Res.* **2004**, *37*, 53-59.

[47] A. E. Pomerantz, O. C. Mullins, G. Paul, J. Ruzicka, M. Sanders. Orbitrap Mass Spectrometry: A Proposal for Routine Analysis of Nonvolatile Components of Petroleum. *Energy Fuels* **2011**, *25*, 3077-3082.

[48] F. H. Field, S. H. Hastings. Determination of Unsaturated Hydrocarbons by Low Voltage Mass Spectrometry. *Anal. Chem.* **1956**, *28*, 1248-1255.

[49] C. S. Creaser, F. Krokos, K. E. O'Neill, M. J. C. Smith, P. G. McDowell. Selective chemical ionization of nitrogen and sulfur heterocycles in petroleum fractions by ion trap mass spectrometry. *J. Am. Soc. Mass. Spectrom.* **1993**, *4*, 322-326.

[50] E. J. Gallegos. Gas Chromatography/Fast-Atom Bombardment Mass Spectrometry of Carboxylic Acids. *J. Chromatogr. Sci.* **1987**, *25*, 296-301.

[51] T. M. Schaub, C. L. Hendrickson, J. P. Quinn, R. P. Rodgers, A. G. Marshall. Instrumentation and Method for Ultrahigh Resolution Field Desorption Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry of Nonpolar Species. *Anal. Chem.* **2005**, *77*, 1317-1324.

[52] Y. Cho, M. Witt, Y. H. Kim, S. Kim. Characterization of crude oils at the molecular level by use of laser desorption ionization Fourier-transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* **2012**, *84*, 8587-8594.

[53] C. J. Lord. Determination of trace metals in crude oil by inductively coupled plasma mass spectrometry with microemulsion sample introduction. *Anal. Chem.* **1991**, *63*, 1594-1599.

[54] C. Robins, P. A. Limbach. The use of nonpolar matrices for matrix-assisted laser desorption/ionization mass spectrometric analysis of high boiling crude oil fractions. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 2839-2845.

[55] P. A. Eckert, P. J. Roach, A. Laskin, J. Laskin. Chemical characterization of crude petroleum using nanospray desorption electrospray ionization coupled with high-resolution mass spectrometry. *Anal. Chem.* **2012**, *84*, 1517-1525.
[56] J. L. Rummel, A. M. McKenna, A. G. Marshall, J. R. Eyler, D. H. Powell. The coupling of direct analysis in real time ionization to Fourier transform ion cyclotron resonance mass spectrometry for ultrahigh-resolution mass analysis. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 784-790.
[57] J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse. Electrospray ionization–principles and practice. *Mass Spectrom. Rev.* **1990**, *9*, 37-70.
[58] D. Zhan, J. B. Fenn. Electrospray mass spectrometry of fossil fuels. *Int. J. Mass spectrom.* **2000**, *194*, 197-208.
[59] J. H. Gross. Mass spectrometry, Wiley Online Library, **2004**.
[60] M. Dole, L. L. Mack, R. L. Hines, R. C. Mobley, L. D. Ferguson, M. B. Alice. Molecular Beams of Macroions. *J. Chem. Phys.* **1968**, *49*, 2240-2249.
[61] J. V. Iribarne, B. A. Thomson. On the evaporation of small ions from charged droplets. *J. Chem. Phys.* **1976**, *64*, 2287-2294.
[62] P. Kebarle. A brief overview of the present status of the mechanisms involved in electrospray mass spectrometry. *J. Mass Spectrom.* **2000**, *35*, 804-817.
[63] G. J. Berkel, S. A. McLuckey, G. L. Glish. Electrochemical Origin of Radical Cations Observed in Electrospray Ionization Mass Spectra. *Anal. Chem.* **1992**, *64*, 1586-1593.
[64] K. Miyabayashi, Y. Naito, K. Tsujimoto, M. Miyake. Structure characterization of polyaromatic hydrocarbons in Arabian mix vacuum residue by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Int. J. Mass spectrom.* **2004**, *235*, 49-57.
[65] D. B. Robb, T. R. Covey, A. P. Bruins. Atmospheric Pressure Photoionization: An Ionization Method for Liquid Chromatography–Mass Spectrometry. *Anal. Chem.* **2000**, *72*, 3653-3659.
[66] A. Raffaelli, A. Saba. Atmospheric pressure photoionization mass spectrometry. *Mass Spectrom. Rev.* **2003**, *22*, 318-331.
[67] D. I. Carroll, I. Dzidic, R. N. Stillwell, M. G. Horning, E. C. Horning. Subpicogram detection system for gas phase analysis based upon atmospheric pressure ionization (API) mass spectrometry. *Anal. Chem.* **1974**, *46*, 706-710.
[68] E. S. de Hoffmann, Vincent Mass Spectrometry: Principles and Applications, John Wiley & Sons, New York, **2007**.
[69] M. B. Comisarow, A. G. Marshall. Fourier transform ion cyclotron resonance spectroscopy. *Chem. Phys. Lett.* **1974**, *25*, 282-283.
[70] A. G. Marshall, C. L. Hendrickson, G. S. Jackson. Fourier transform ion cyclotron resonance mass spectrometry: A primer. *Mass Spectrom. Rev.* **1998**, *17*, 1-35.
[71] C. L. Hendrickson, J. P. Quinn, N. K. Kaiser, D. F. Smith, G. T. Blakney, T. Chen, A. G. Marshall, C. R. Weisbrod, S. C. Beu. 21 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer: A National Resource for Ultrahigh Resolution Mass Analysis. *J. Am. Soc. Mass. Spectrom.* **2015**, *26*, 1626-1632.
[72] K. O. Nagornov, M. V. Gorshkov, A. N. Kozhinov, Y. O. Tsybin. High-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry with Increased Throughput for Biomolecular Analysis. *Anal. Chem.* **2014**, *86*, 9020-9028.
[73] W. Schrader, H. W. Klein. Liquid chromatography/Fourier transform ion cyclotron resonance mass spectrometry (LC-FTICR MS): an early overview. *Anal. Bioanal. Chem.* **2004**, *379*, 1013-1024.

[74] A. Makarov. Electrostatic axially harmonic orbital trapping: a high-performance technique of mass analysis. *Anal. Chem.* **2000**, *72*, 1156-1162.
[75] Q. Hu, R. J. Noll, H. Li, A. Makarov, M. Hardman, R. Graham Cooks. The Orbitrap: a new mass spectrometer. *J. Mass Spectrom.* **2005**, *40*, 430-443.
[76] A. Makarov, E. Denisov, O. Lange. Performance Evaluation of a High-field Orbitrap Mass Analyzer. *J. Am. Soc. Mass. Spectrom.* **2009**, *20*, 1391-1396.
[77] J. E. P. Syka, J. A. Marto, D. L. Bai, S. Horning, M. W. Senko, J. C. Schwartz, B. Ueberheide, B. Garcia, S. Busby, T. Muratore, J. Shabanowitz, D. F. Hunt. Novel Linear Quadrupole Ion Trap/FT Mass Spectrometer: Performance Characterization and Use in the Comparative Analysis of Histone H3 Post-translational Modifications. *J. Proteome Res.* **2004**, *3*, 621-626.
[78] A. Makarov, E. Denisov, A. Kholomeev, W. Balschun, O. Lange, K. Strupat, S. Horning. Performance Evaluation of a Hybrid Linear Ion Trap/Orbitrap Mass Spectrometer. *Anal. Chem.* **2006**, *78*, 2113-2120.
[79] W. Paul, H. Steinwedel. Ein neues Massenspektrometer ohne Magnetfeld. *Z. Naturforsch. A* **1953**, *8*, 448-450.
[80] W. Paul. Electromagnetic traps for charged and neutral particles. *Rev. Mod. Phys.* **1990**, *62*, 531-540.
[81] R. E. March. An Introduction to Quadrupole Ion Trap Mass Spectrometry. *J. Mass Spectrom.* **1997**, *32*, 351-369.
[82] J. C. Schwartz, M. W. Senko, J. E. P. Syka. A two-dimensional quadrupole ion trap mass spectrometer. *J. Am. Soc. Mass. Spectrom.* **2002**, *13*, 659-669.
[83] D. J. Douglas, A. J. Frank, D. Mao. Linear ion traps in mass spectrometry. *Mass Spectrom. Rev.* **2005**, *24*, 1-29.
[84] M. E. Belov, R. Zhang, E. F. Strittmatter, D. C. Prior, K. Tang, R. D. Smith. Automated Gain Control and Internal Calibration with External Ion Accumulation Capillary Liquid Chromatography-Electrospray Ionization-Fourier Transform Ion Cyclotron Resonance. *Anal. Chem.* **2003**, *75*, 4195-4205.
[85] E. de Hoffmann. Tandem mass spectrometry: A primer. *J. Mass Spectrom.* **1996**, *31*, 129-137.
[86] R. A. Yost, C. G. Enke. Triple Quadrupole Mass Spectrometry. *Anal. Chem.* **1979**, *51*, 1251A-1264A.
[87] J. N. Louris, R. G. Cooks, J. E. P. Syka, P. E. Kelley, G. C. Stafford, J. F. J. Todd. Instrumentation, applications, and energy deposition in quadrupole ion-trap tandem mass spectrometry. *Anal. Chem.* **1987**, *59*, 1677-1685.
[88] L. Sleno, D. A. Volmer. Ion activation methods for tandem mass spectrometry. *J. Mass Spectrom.* **2004**, *39*, 1091-1112.
[89] A. K. Shukla, J. H. Futrell. Tandem mass spectrometry: dissociation of ions by collisional activation. *J. Mass Spectrom.* **2000**, *35*, 1069-1090.
[90] P. K. Taylor, I. J. Amster. Space charge effects on mass accuracy for multiply charged ions in ESI–FTICR. *Int. J. Mass spectrom.* **2003**, *222*, 351-361.
[91] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah. Mechanistic investigation of ionization suppression in electrospray ionization. *J. Am. Soc. Mass. Spectrom.* **2000**, *11*, 942-950.
[92] H. Trufelli, P. Palma, G. Famiglini, A. Cappiello. An overview of matrix effects in liquid chromatography–mass spectrometry. *Mass Spectrom. Rev.* **2011**, *30*, 491-509.
[93] P. Schmitt-Kopplin, M. Englmann, R. Rossello-Mora, R. Schiewek, K. J. Brockmann, T. Benter, O. J. Schmitz. Combining chip-ESI with APLI (cESILI) as a multimode

source for analysis of complex mixtures with ultrahigh-resolution mass spectrometry. *Anal. Bioanal. Chem.* **2008**, *391*, 2803-2809.

[94] M. B. Mieczyslaw, in *Analytical Methods in Petroleum Upstream Applications*, CRC Press, **2015**, pp. 3-30.

[95] E. Kendrick. A Mass Scale Based on CH2 = 14.0000 for High Resolution Mass Spectrometry of Organic Compounds. *Anal. Chem.* **1963**, *35*, 2146-2154.

[96] W. C. Hockaday, A. M. Grannas, S. Kim, P. G. Hatcher. Direct molecular evidence for the degradation and mobility of black carbon in soils from ultrahigh-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil. *Org. Geochem.* **2006**, *37*, 501-510.

[97] D. W. van Krevelen. Graphical-statistical method for the study of structure and reaction processes of coal. *Fuel* **1950**, *29*, 269-284.

[98] S. Kim, R. W. Kramer, P. G. Hatcher. Graphical Method for Analysis of Ultrahigh-Resolution Broadband Mass Spectra of Natural Organic Matter, the Van Krevelen Diagram. *Anal. Chem.* **2003**, *75*, 5336-5344.

CHAPTER 2.

ELECTROSPRAY IONIZATION FOR DETERMINATION OF NON-POLAR POLYAROMATIC HYDROCARBONS AND POLYAROMATIC HETEROCYCLES IN HEAVY CRUDE OIL ASPHALTENES

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2.1 Abstract

Electrospray ionization (ESI) is the most common ionization method in atmospheric pressure ionization mass spectrometry because of its easy use and handling and because a diverse range of components can be effectively ionized from high to medium polarity. Usually, ESI is not employed for the analysis of non-polar hydrocarbons, but under some circumstances, they are effectively ionized. Polyaromatic hydrocarbons and aromatic heterocycles can form radical ions and protonated molecules after ESI, which were detected by Fourier transform ion cyclotron resonance mass spectrometry. The highly condensed aromatic structures are obtained from a heavy crude oil, and the results show class distribution from pure hydrocarbons up to more non-basic nitrogen-containing species. By using different solvent compositions (toluene/methanol (50/50 v/v), dichloromethane/methanol (50/50 v/v), dichloromethane/acetonitrile (50/50 v/v) and chloroform), the results show that the lack of proton donor agent helps to preserve the radical formation that was created at the metal/solution interface inside the electrospray capillary. The results demonstrate that with an appropriate selection of solvent and capillary voltage, the ratio between the detected radical ion and protonated molecule form can be manipulated. Therefore, ESI can be expanded for the investigation of asphaltene and other polyaromatic systems beyond the polar constituents as non-polar hydrocarbons can be efficiently analyzed.

2.2 Introduction

Although research efforts to develop new renewable energy resources strongly increased in the last years, crude oil sources have received special interest because crude oil is a necessary resource until sustainable materials are available in the desired amounts. In order to sustain the increasing need for energy supply for the future, more viscous and dirtier crude oil resources, like oil shale and tar sands, will be used for upgrading into petroleum.[1] However, this involves a number of additional problems during oil transportation, separation and refining processes. Most of them are associated with asphaltenes.[2] According to their definition, the asphaltene fraction of crude oil is insoluble in paraffinic solvents (e.g. *n*-heptane) and soluble in toluene. They possess large and very highly aromatic structures causing some problematic features such as limited solubility, indistinct structure formation, aggregation formation tendency and complexity.[3-5]

Regarding their chemical composition and structure, they are a heterogeneous complex mixture with polycondensed aromatic hydrocarbon rings and a relatively large number of heteroatoms (N, O and S) and metals (V and Fe) present, depending on the origin of crude oil. Their average features can be characterized by different analytical methods; however, for investigations on a molecular level, mass spectrometry (MS) has been developed into the method of choice.[6-12]

Mass spectrometry with ultrahigh resolution and high mass accuracy such as Fourier transform ion cyclotron resonance (FT-ICR) MS is an appropriate tool to provide sufficient information about the individual elemental composition ($C_cH_hN_nO_oS_s$) of a complex sample, because it allows to differentiate thousands of species within a single mass spectrum.[13] The combination of MS and chromatographic separation methods or extraction processes can help to explore more information about the structural complexity.[14, 15]

Asphaltenes contain molecules with highly conjugated structures, which can be efficiently ionized by sources using UV radiation such as atmospheric pressure photoionization and atmospheric pressure laser ionization but are more difficult to analyze with any other ionization method.[8]

Electrospray ionization (ESI) is primarily used for determination of polar heteroatom-containing compounds such as proteins,[16, 17] which are detected in the

form of $[M + nH]^{n+}$, where n means the number of charges and depends on the polar sites of the molecule. However, some reports can be found describing that electrospray is able to ionize non-polar compounds with more than two condensed rings in radical ion form.[18-20] Miyabayashi and co-workers have reported that by using model compounds in methanol/chloroform solvent mixture, nitrogen-containing compounds and aromatic hydrocarbons can be detected at even *m/z* values in mass spectra by ESI. Although in a mixture of equal molar concentration of standards, mostly basic nitrogen components were detectable as protonated molecules. Decreasing the concentration of nitrogen compounds (to 0.4 wt.%), their intensity was roughly the same as it was for hydrocarbon molecular radical ions. In their study, they have reported that for the prosperous detection and distinction of these components in complex samples, such as Arabian mix vacuum residue, addition of trifluoracetic acid was necessary to the solvent system to help the protonation of hydrocarbons.

Asphaltenes are considered to contain the highest number of polar compounds, and for a full representation of a crude oil sample, different ionization methods are necessary.[8] During our experiments while studying complex asphaltenes, our results showed that ESI is not only suitable for determination of polar compounds but also for detection of polyaromatic hydrocarbons. It was surprising that the data received by ESI were not restricted to the usual suspects, such as polyaromatic heterocycles containing only polar groups such as nitrogen. In addition, pure hydrocarbons appeared frequently or in some cases also, compounds containing sulfur atoms. In our experiments, these compounds were detected in radical ion form. The appearance of these low-polar or non-polar compound classes needs further investigation. Therefore, here we studied the outcome of asphaltene analysis by electrospray MS.

2.3 Experimental section

2.3.1 Data analysis

Asphaltenes were precipitated from a heavy crude using *n*-heptane [high-performance liquid chromatography (HPLC) grade, Merck, Germany]. During their preparation, a modified IP 143 method was applied.[21] Briefly, 30 ml of *n*-heptane per g of crude oil was added and then mixed to obtain a fully dispersed solution. The mixture was refluxed for 1 h, then cooled down and stored in the dark overnight. The

precipitated particles were filtered through a filter paper (Whatman, type 589.3). The sample was rinsed with hot heptane then the sample with the filter paper was placed into a Soxhlet apparatus and extracted under reflux with *n*-heptane for 2 h. Then, the solvent was replaced with fresh toluene (HPLC grade, Sigma-Aldrich, Germany), and the extraction proceeded until asphaltenes had been dissolved from the filter paper (20 h). The toluene was removed by evaporation under vacuum.

Further chemicals used for sample preparation and model compounds were purchased from Sigma-Aldrich (Germany), except methanol (HPLC grade, Merck, Germany).

For FT-ICR MS analysis, asphaltene (4 mg) was dissolved in 4 ml of the following solvents: toluene, dichloromethane and chloroform. For electrospray measurements, these stock solutions were further diluted with methanol and acetonitrile. A final concentration of 400 μg/ml was obtained for injection. The same stock solutions were made for model compounds such as anthracene, fluorene, 9-phenylcarbazole and coronene. In every case, a mixture was prepared containing the same molar concentration of each standard compound and the desired solvent composition was achieved by addition of methanol or acetonitrile.

Agilent ESI Tuning Mix calibration solution was added to the solutions as an internal standard before analysis.

2.3.2 Mass spectrometry

Mass spectrometric analysis was performed on a 7 T linear trap quadrupole (LTQ) FT-ICR mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with ESI source. The data were collected and processed with LTQ FT Ultra 2.5.5 (Thermo Fisher Scientific, Bremen, Germany) data system. The sample solutions were infused at a flow rate of 5 μl/min, and the ionization was performed with a stainless steel needle with 4 kV needle voltage in positive mode. In some experiments, the metal needle was replaced with a fused silica capillary. Nebulization was assisted by a sheath gas flow of 4 (arbitrary units). Furthermore, auxiliary gas flow and sweep gas flow were both adjusted to 1 (arbitrary unit), while the transfer capillary was set at a temperature of 275 °C with a capillary voltage of 35 V. The spectra were acquired in positive mode in the mass range of *m/z* 150–1000. The mass resolution for FT-ICR MS was set to 400 000

(at *m/z* 400). The ion accumulation time was adjusted by the automatic gain control, and it was set to 500 000.[22]

2.3.3 Data analysis

The acquired data were analyzed by Xcalibur 2.2 software (Thermo Fischer Scientific, Bremen, Germany). Additionally, the peak lists were imported and converted to molecular formulas by Composer V1.0.5 software (Sierra Analytics, Modesto, CA, USA). The applied parameters and chemical constraints were the following: $0 < H < 1000$, $0 < C < 200$, $0 < N < 3$, $0 < O < 3$, $0 < S < 3$ and $0 < DBE < 40$ with a maximum mass error of 1 ppm. The assignments of the most abundant ions were confirmed through their isotopic peaks. Radical cations and protonated molecules were distinguished and assigned separately (X, X[H]). The results with the calculated molecular formulae were sorted into compound classes. The received mass lists were transferred into Excel (Microsoft Office Professional Plus 2010, Microsoft Excel 2010) for further data evaluation.

2.4 Results and discussion

2.4.1 Characterization of asphaltenes by ESI

During the ionization of asphaltenes by electrospray, signals from ions of polyaromatic hydrocarbons and nitrogen-containing polyaromatic heterocycles were simultaneously present in the mass spectra. Hydrocarbons were detected only as radical cations, while nitrogen-containing heterocycles appear both as protonated and radical cations with high intensity in the complex mixture containing highly polycondensed aromatic compounds. Our first observation regarding the presence of radical cations in electrospray source was that using toluene/methanol (50/50 v/v) solvent as the most common solvent system for asphaltene samples for electrospray,[23] species containing one nitrogen atom were detectable not only at even *m/z* values (as protonated molecules) but also at odd *m/z* values (as radical cations) (Figure 2-1, bottom right, e.g. $C_{34}H_{19}N$ at *m/z* 441.15121). The full positive ESI FT-ICR mass spectrum of the asphaltene sample is shown in Figure 2-1 (top) and mass scale-expanded views at *m/z* 440 and 441 (bottom left and right) with the assignment of HC, N and N_2 compounds.

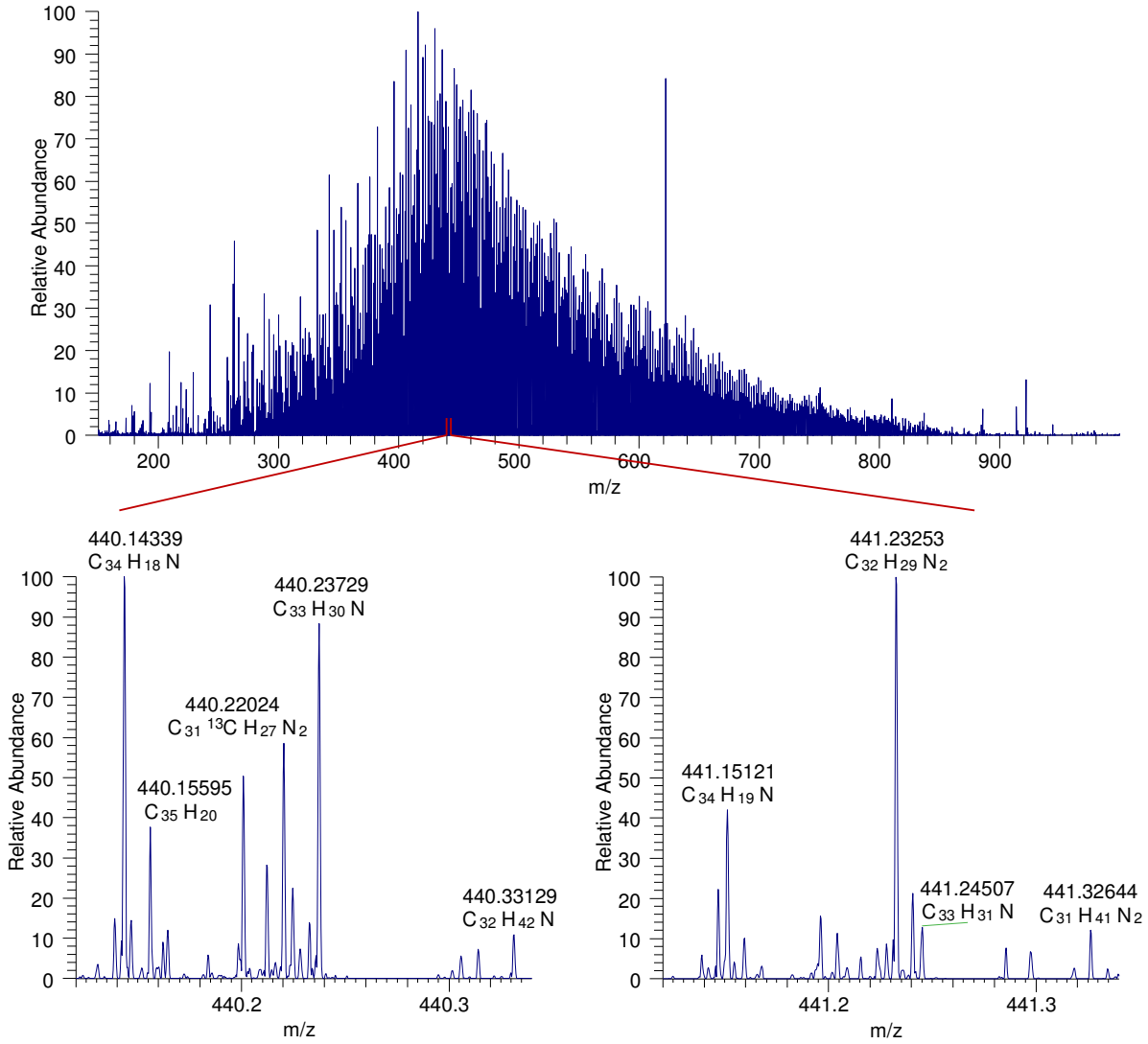

Figure 2-1. Full mass range positive electrospray ionization Fourier transform ion cyclotron resonance mass spectrum of asphaltenes in toluene/methanol (50/ 50 v/v) (top) and mass scale-expanded views of the full mass range (bottom) with the assignment of HC, N and N_2 compounds. For example, a nitrogen-containing compound (containing one nitrogen atom, $C_{34}H_{19}N$) and a hydrocarbon ($C_{35}H_{20}$) at *m/z* 441.15121 (odd *m/z* value) and *m/z* 440.15595, respectively, were detected in radical cation form.

The presence of radical cations was not only observed in case of nitrogen-containing species, but also hydrocarbons were successfully detected as radical cations (Figure 2-1, bottom left, $C_{35}H_{20}$ at *m/z* 440.15595), and their isotopic patterns were also discernible within the mass spectra. This observation can extend the utility of ESI for pure hydrocarbons.

All the data obtained from the ESI spectrum can be converted into elemental compositions and classified into the respective compound class. Especially helpful for the characterization is the double bond equivalents (DBE) value, which can give an indication about the aromaticity because it describes the number of double bonds and

ring closing bonds in a molecule. In Figure 2-2, the calculations of the ESI spectrum are summarized in a heatmap, which is highlighting the large DBE of the most abundant classes in positive ESI FT-ICR mass spectra of asphaltenes.

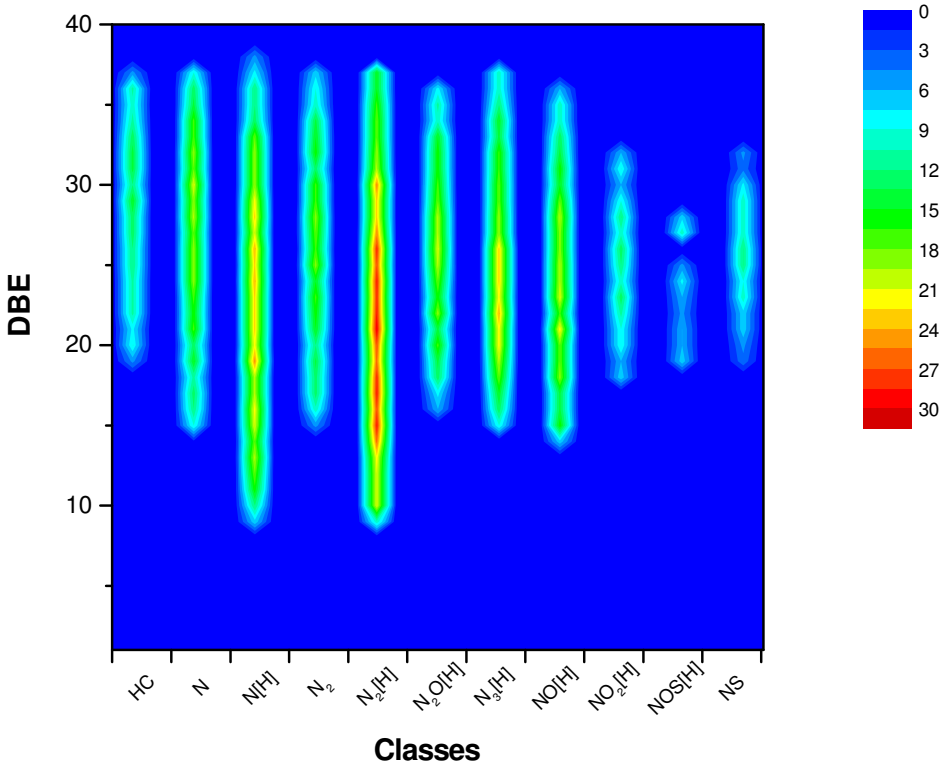

Figure 2-2. Double bond equivalent (DBE) distribution of the most abundant classes of asphaltenes in toluene/methanol (50/50 v/v) (in the third axis scaled according to population). Protonated species are depicted as [H], while radical cations are shown without the bracket.

Asphaltenes have a high abundance of species containing high number of rings plus double bonds.[6] However, there is a difference in the range of DBE numbers between the species detected in their protonated and radical cation form, especially for N and N_2 compounds. The DBE number for protonated species (N[H] and N_2[H]) is in the range of 9–38 with the highest abundance between 12 and 30. This value range for species assigned as radical cations from 20 to 38 (for HC-species) and from 15 to 38 (for N-species and N_2-species). These high DBE values refer to their extremely high aromatic structure. The degree of aromaticity can also be illustrated by the normalization of DBE to the number of carbon atoms (DBE/C) for each assigned compound in the function of *m/z* values as Figure 2-3 shows. This representation was applied successfully by Hockaday and co-workers to identify species with condensed aromatic ring structures (CARS) in investigation of degradation of charcoal in soils.[24] A threshold of 0.7 for

DBE/C value can specify CARS.[25] More than 0.7 of DBE/C value can only be reached by polycyclic aromatic compounds and the maximum upper limit is 0.9. Comparison of the diagrams in Figure 2-3 shows that there is a difference between the species detected in protonated and radical cation form for heavy crude oil asphaltenes. While the DBE/C range for N[H] and N_2[H] is from 0.3 to ~0.85 (Figure 2-3, right), this range for the radical HC-species, N-species and N_2-species was narrower, from 0.5 to ~0.85 (Figure 2-3, left), so around half of these latter type of compounds belong to CARS.

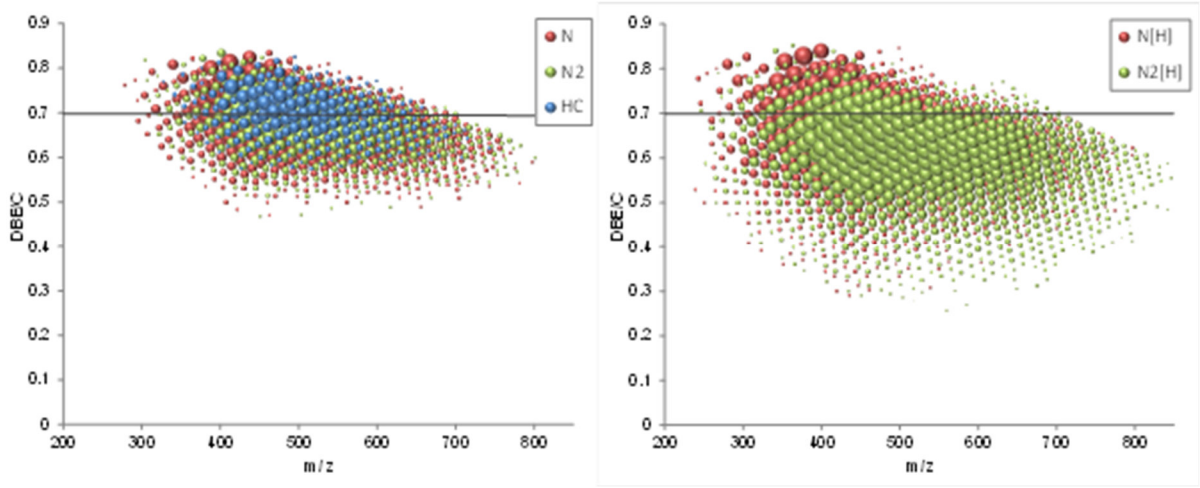

Figure 2-3. Double bond equivalents to the number of carbon atoms (DBE/C) versus *m/z* for HC, N and N_2 compounds detected in radical cation form (left) and protonated molecule form (right). The criterion is 0.7 for identifying species with condensed aromatic ring structure.

As these results show, detection of both protonated and radical cations was accomplished by ESI. Species detected in their radical cation forms (non-polar hydrocarbons and non-basic nitrogen-containing compounds) seem to have exclusively high aromaticity compared with the protonated ones, which also contain compounds with those features but others with lower aromaticity, as well. The more polar nitrogen-containing compounds are, in general, the less difficult to ionize under ESI conditions. The non-polar hydrocarbons are forming radical cations especially from the highly condensed species above the 0.7 line, while the lower condensed (below 0.5) and less aromatic nitrogen species are only forming protonated molecules.

2.4.2 Investigation of different solvent systems in determination of polycondensed structures by ESI

Characterization of asphaltenes in different solvent systems

The unusual phenomenon, namely observation of radical cations in ESI was further investigated. Berkel and co-workers confirmed that the electrospray ion source operates as a controlled-current electrolytic flow cell.[26] In addition to the applied electric field, the type of solvent can have a great influence on the ionization process in creating either protonated molecules ($[M + H]^+$) or radical cations ($M^{\bullet+}$), which means that using an appropriate solvent is apparently one of the key steps during the analysis of radical cations.[18]

Different solvent systems (containing protic and aprotic components) have been chosen to examine their effect to the radical cation lifetime. Besides, the mixture of toluene/methanol (50/50 v/v), dichloromethane/methanol (50/50 v/v), dichloromethane/ acetonitrile (50/50 v/v), chloroform and toluene have been selected. Using only toluene did not produce a stable spray, but in all of the other cases, the differences can be observed. Figure 2-4 shows an expanded view of mass scale of the full scan positive ESI FT-ICR mass spectrum in case of the aforementioned solvent systems and the assignments of the peaks highlighting the hydrocarbons, the N and N_2 compounds. It is remarkable that at an even *m/z* value (in the range of *m/z* 554.18-554.40), hydrocarbons ($C_{44}H_{26}$ and $C_{43}H_{38}$) and N_2 compounds ($C_{41}H_{34}N_2$ and $C_{40}H_{46}N_2$) are assignable as radical cations, and N compounds ($C_{43}H_{24}N$, $C_{42}H_{36}N$ and $C_{41}H_{48}N$) are in their protonated form in this range, but with different intensities for each solvent.

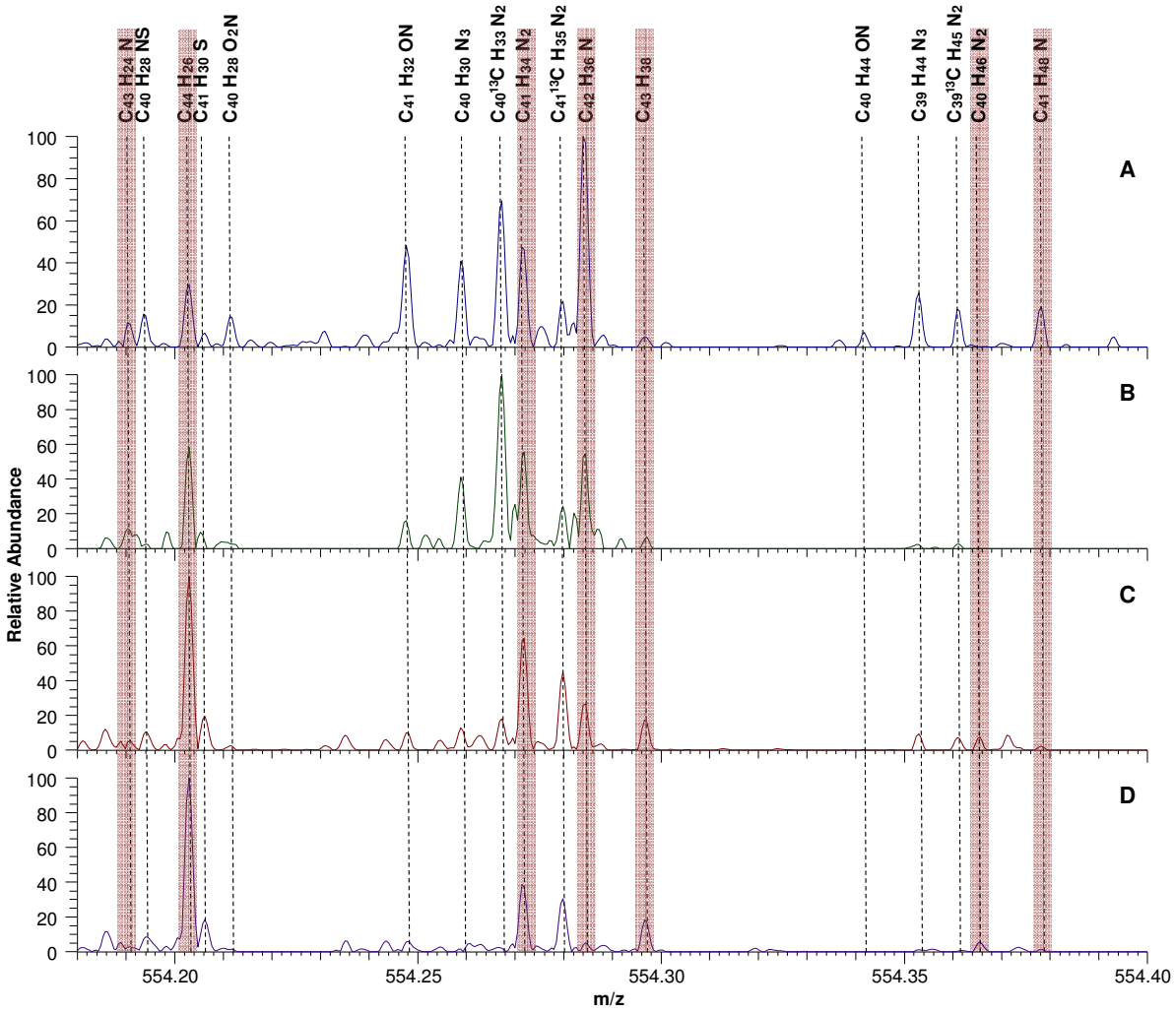

Figure 2-4. Mass scale-expanded view of full scan positive electrospray ionization Fourier transform ion cyclotron resonance mass spectra of asphaltenes using different solvent systems with the assignments of the peaks highlighting the hydrocarbons, the N and N_2 compounds. (A) Toluene/methanol (50/ 50 v/v); (B) Dichloromethane/methanol (50/50 v/v); (C) Dichloromethane/acetonitrile (50/50 v/v); (D) Chloroform.

HC, N and N_2 compounds are the most abundant assignable peaks with high intensity in the whole mass spectra. In Figure 2-5, the intensity-based distributions of these compounds (both radical and protonated forms) in case of different solvent systems are summarized.

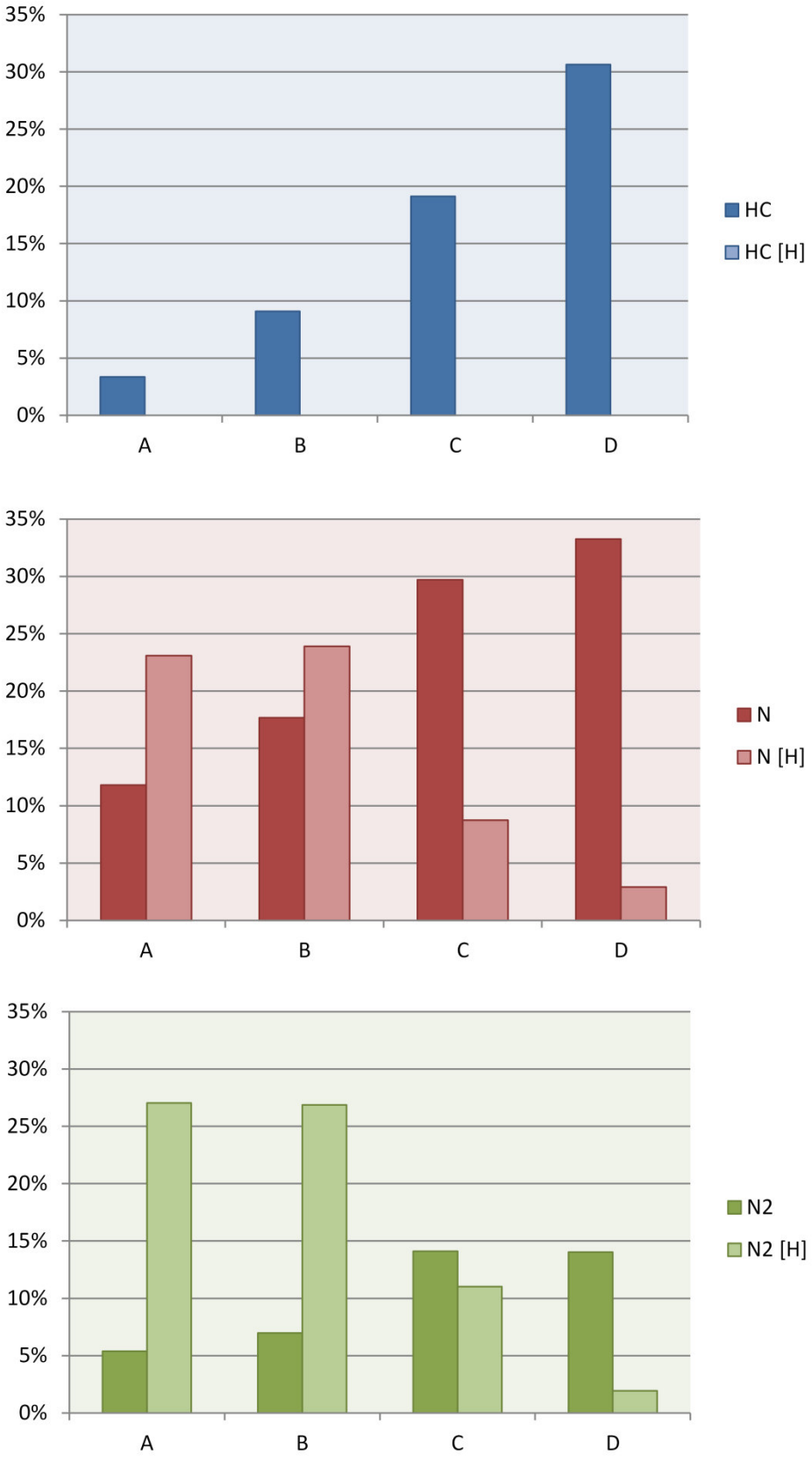

Figure 2-5. Comparison of intensity-based distribution of HC, N and N_2 compounds (both radical and protonated [H] forms) with different solvent systems for asphaltenes. (A) Toluene/methanol (50/50 v/v); (B) Dichloromethane/methanol (50/50 v/v); (C) Dichloromethane/acetonitrile (50/50 v/v); (D) Chloroform.

The presence of protic solvent systems can help the neutral molecules to be ionized creating protonated cations. The capability to attach a proton to the analyte ($M + H^+ \rightarrow [M + H]^+$) is decreasing from solvent system A to D. This can be correlated with the pK_a values of the solvent systems. For example, using dichloromethane/acetonitrile (C) instead of dichloromethane/ methanol (B) can create less protonated molecules because pK_a of acetonitrile (p$K_{a,acetonitrile}$=25)[27] is higher than pK_a of methanol (p$K_{a,methanol}$=15.5).[28] Because of the diminishing proton donor capabilities from solvent system A to D, the facility to protect the generated radical cations by the electrolysis process is increasing. Using chloroform (D), the presence of radical cations (HC, N and N2 in Figure 2-5) increased significantly compared with their corresponding protonated ions. According to our observations, the ionization process is strongly governed by the solvent system itself. With the appropriate selection of solvents, the number of radical cations can be enhanced. This phenomenon requires that the analyte release an electron very easily [low ionization energy (IE)], which is generally the case for highly aromatic compounds. IE of the compounds refers to the ability to give up the most easily removable electron and create a radical cation. Therefore, IE can be an indication to predict the behavior of the molecule.

Moreover, appropriate ESI conditions such as sufficient ESI current and available metal/solution interface for the analyte need to be present.[29, 30] Determination of hydrocarbons and nitrogen species as radical cations in ESI is rather uncommon in MS. Therefore, we performed the verification by recording model compounds, which will be discussed in more detail in the following.

Investigation of ionization efficiencies of model compounds with different solvent systems

Detection of polycyclic aromatic hydrocarbons with ESI has been reported before.[18-20] In our study, some model compounds, hydrocarbons (anthracene, fluorene and coronene) and non-basic nitrogen-containing compound (9-phenylcarbazole), were investigated to obtain a better insight into the ionization process of these compounds (Table 2-1).

Table 2-1. Monoisotopic mass and ionization energy of model compounds

Compound	Molecular formula	Monoisotopic mass (Da)	Ionization energy (eV)
Anthracene	$C_{14}H_{10}$	178.07770	7.45[31]
Fluorene	$C_{13}H_{10}$	166.07770	7.89[31]
9-phenylcarbazole	$C_{18}H_{13}N$	243.10425	7.7[32]
Coronene	$C_{24}H_{12}$	300.09335	7.29[31]

Both radical cations and protonated molecules were detected with different intensity ratio for anthracene, 9-phenylcarbazole and coronene by using dichloromethane/methanol (50/50 v/v) (Figure 2-6). For fluorene, no peak was observed. These results are in good agreement with Miyabayashi's statement that compounds with IE lower than 7.86 eV can be detected in their radical cation form by ESI.[20]

For determination of the formation of radical cations and protonated molecules, mixtures of these model compounds were prepared using the same solvent systems as for the asphaltene sample [toluene/methanol (50/50 v/v), dichloromethane/methanol (50/50 v/v), dichloromethane/acetonitrile (50/50 v/v) and chloroform]. In case of coronene (Figure 2-6, bottom), which has high similarity to the components in asphaltene sample with its seven condensed aromatic rings (DBE=19), the radical cation peak ($C_{24}H_{12}$) was stronger compared to the protonated one ($C_{24}H_{13}$), and in the solvent systems lacking methanol as proton donor, only the radical cation peak and its ^{13}C isotope peak were detected (Figure 2-7(D)).

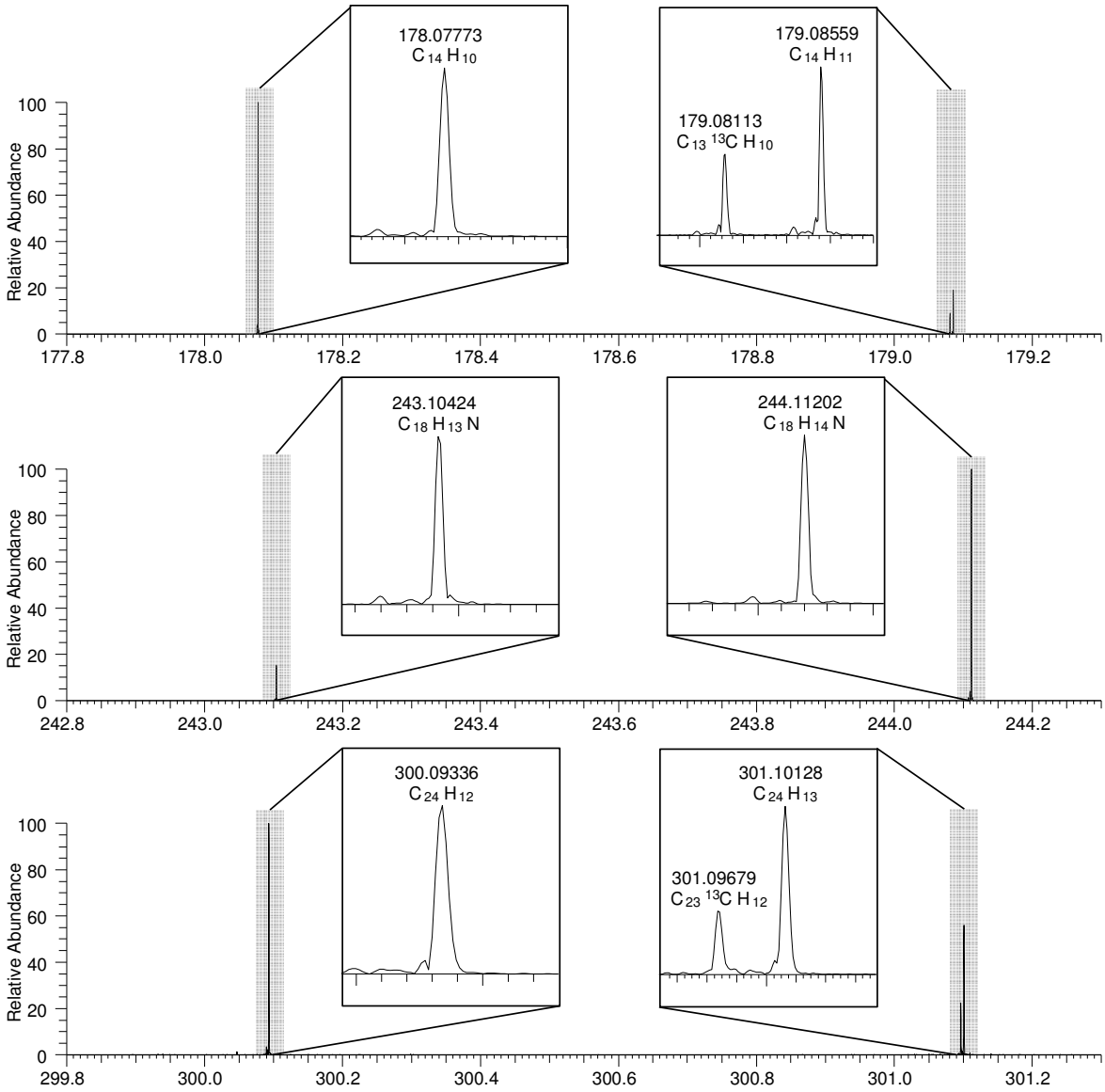

Figure 2-6. Positive electrospray ionization Fourier transform ion cyclotron resonance mass spectra of model compounds in dichloromethane/methanol (50/ 50 v/v); radical cations and protonated molecules were detected simultaneously for anthracene (top, *m/z* 178.07773 and 179.08559), for 9-phenylcarbazole (center, *m/z* 243.10424 and 244.11202) and for coronene (bottom, *m/z* 300.09336 and 301.10128).

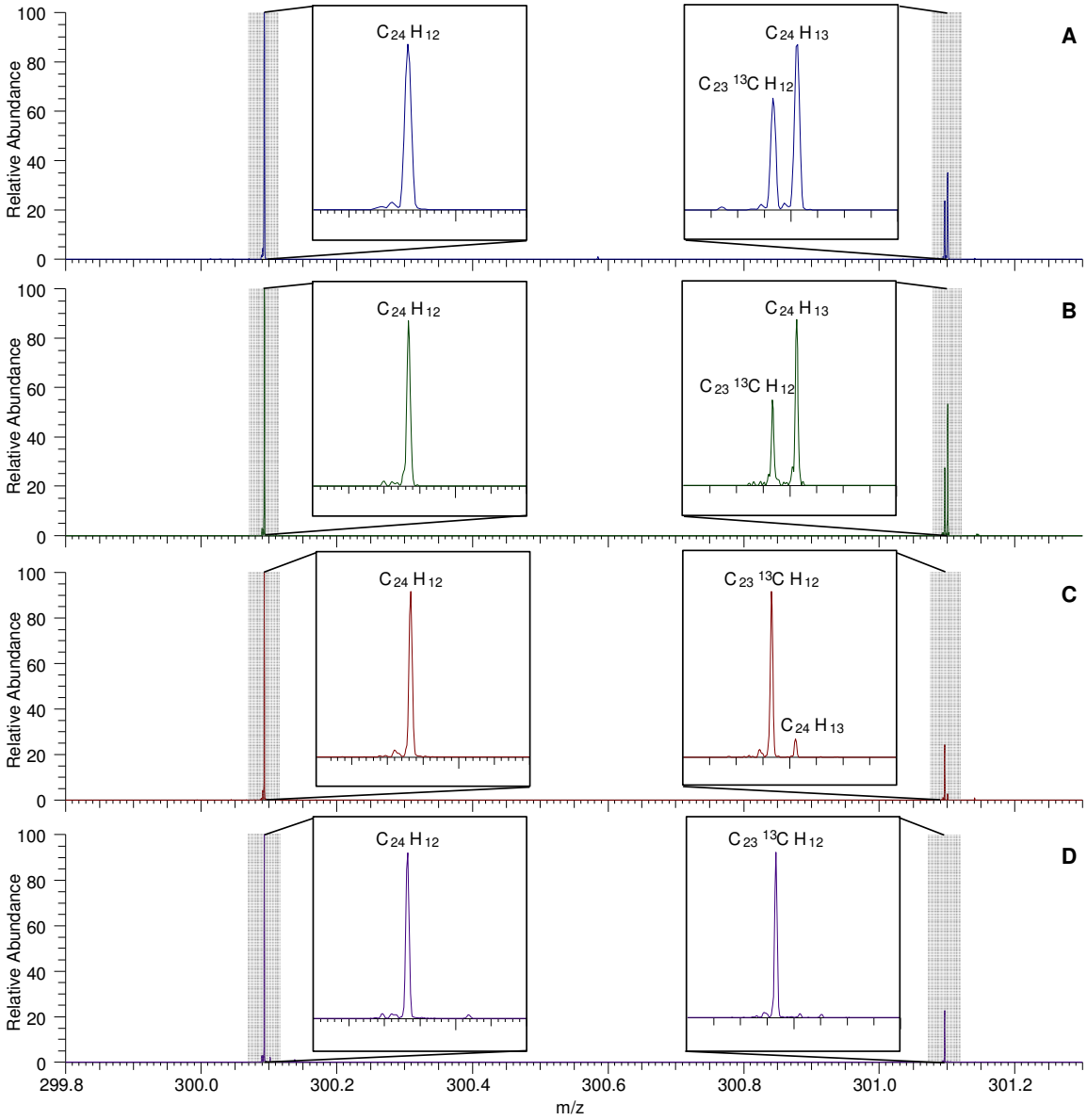

Figure 2-7. Positive electrospray ionization Fourier transform ion cyclotron resonance mass spectra of coronene using different solvent systems. (A) Toluene/ methanol (50/50 v/v); (B) Dichloromethane/methanol (50/50 v/v) (C) Dichloromethane/acetonitrile (50/50 v/v); (D) Chloroform. Radical cation peak ($C_{24}H_{12}$), its isotope peak ($C_{23}{}^{13}CH_{12}$) and protonated molecule peak ($C_{24}H_{13}$) was detected.

2.4.3 Investigation of ESI setup and capillary voltage

For observation of radical cations under ESI, adequate conditions are necessary. Berkel and co-workers demonstrated that the setup of the ion source is very important. Especially, the type of the capillary has a big role in producing the different types of ions.[18] During the aforementioned experiments, a stainless steel capillary was used in the ESI nebulizer. In an additional experiment, the metal needle was replaced with fused silica capillary, and only insulating plastic connections were used in order to avoid any

direct metal/solution contact and the occurrence of electrochemical reactions. In this case, it was found that the radical cation peak ($C_{24}H_{12}$) was still present (Figure 2-8(A)) but with much lower intensity than it was observed with the metal needle setup (Figure 2-8(B)). The modification of ESI setup affects ion formation in the spectra. Therefore, the type of capillary inside the ESI source is important for the electrochemistry, which is in accordance to the report from Berkel and co-workers. However, as the results show, in formation of radical cations, not only an electrochemical reaction can take place. The presence of radical cation without the chance of electrochemical reaction (so the aforementioned application of fused silica capillary) refers to the chemical oxidation via charge transfer between the analyte and the solvent components especially for very aromatic species having low IE where the radical cation is being stabilized by the π-electron system. This can confirm the importance of the solvent system that plays a major role in creating radical cations prior to the spraying process.

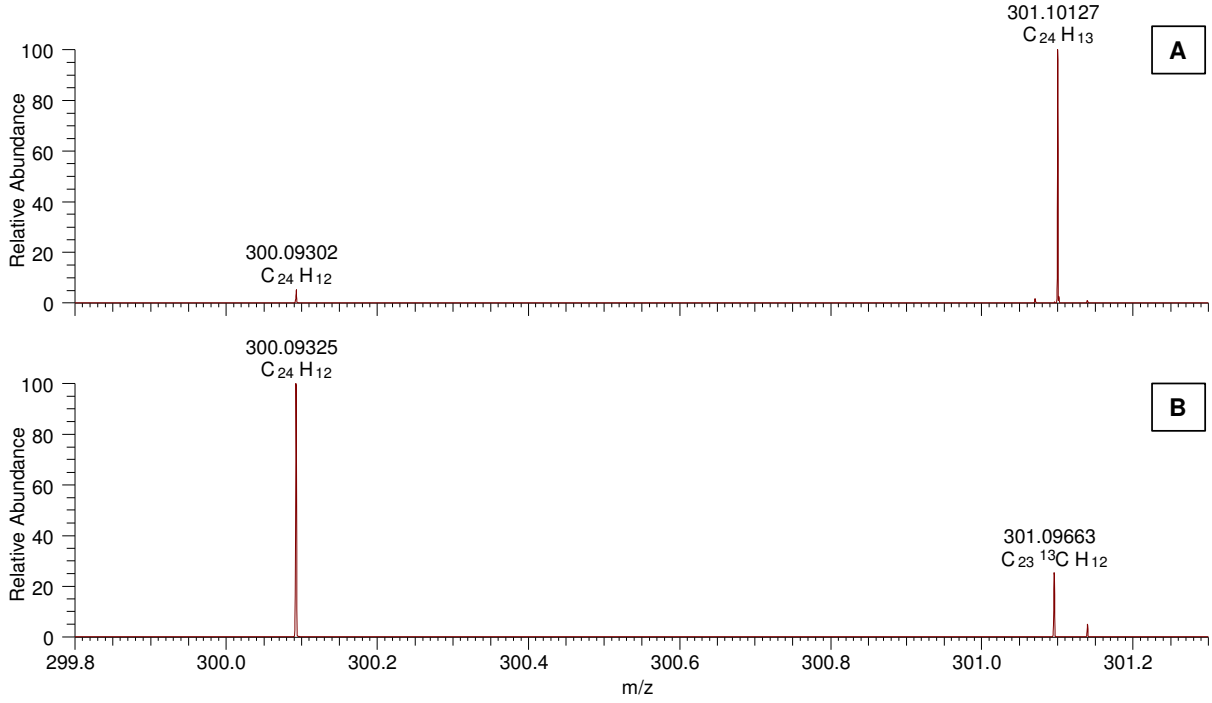

Figure 2-8. Positive electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectra of coronene in dichloromethane/acetonitrile (50/50 v/ v). (A) ESI setup with fused silica capillary; (B) ESI setup with stainless steel capillary.

Furthermore, the effect of applied capillary voltage was investigated. The intensity ratio between the detected radical cation peaks and protonated molecule peaks ($R_{radical/protonated}$) in each case of standards is represented as function of the capillary voltage (in the range of 3.5–5.0 kV) in Figure 2-9. It was found that in case of coronene,

the intensity of the radical cation peak increases with increasing voltage. The effect of increasing voltage is not that significant in case of anthracene. For 9-phenylcarbazole, the protonated molecule is more abundant with higher voltage (the ratio is lower than 1.0, and it is decreasing slightly with increasing voltage).

The results suggest that the intensity of radical cations and protonated molecules can be slightly manipulated with the help of the ionization potential. The intensity of radical cations from polyaromatic hydrocarbons can be enhanced with increasing voltage.

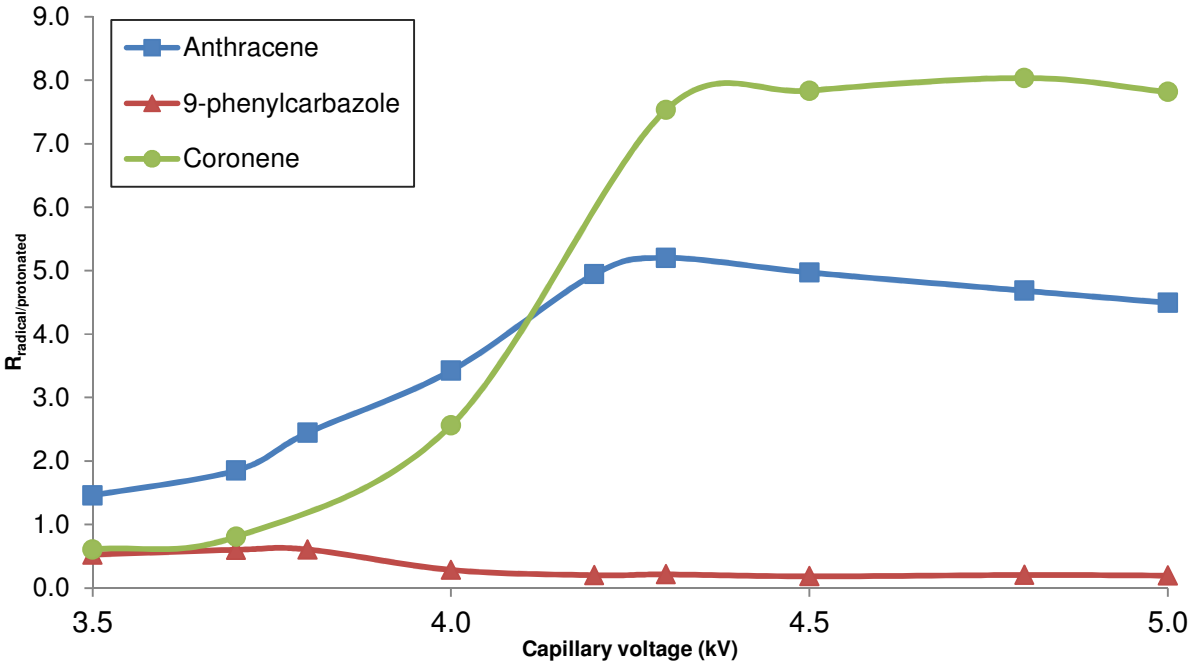

Figure 2-9. Intensity ratio between radical cation peaks and protonated molecule peaks ($R_{radical/protonated}$) of model compounds (anthracene, 9-phenylcarbazole and coronene) in the function of capillary voltage with dichloromethane/methanol (50/50 v/v).

Overall, the results indicate that an electrolytic process is taking place inside the capillary achieving charge balance. While forming the positive charged droplets at the tip of the capillary, oxidation can occur in the capillary at the metal/solution interface with the analyte in solution creating radical cations by releasing an electron

$$(M - e^- \rightarrow M^{\bullet+})$$

which is in accordance with result already reported in the literature.[18, 29, 30, 33, 34]

Radical cations can be created in solution in the sprayer via charge-transfer complexation, as well.[35] An electron transfer can happen between an electron donor

(D) agent and an electron acceptor (A) agent where a charge-transfer complex is created during the reaction

$$D + A \rightarrow (D^{\bullet+}A^{\bullet-}) \rightarrow (D^{\bullet+})\text{solvate} + (A^{\bullet-})\text{solvate}$$

Depending on the feature of the solvent system, compounds having an easily removable electron can undergo this type of rapid reaction in solution and create radical cations, while the presence of a proton donating solvent can lead to the formation of a protonated molecule.[36] Therefore, if radical ions need to be preserved, protic solvents and nucleophilic additives should be avoided.

2.5 Conclusion

Against conventional wisdom, ESI is not only a method to ionize polar components. It could be demonstrated that polyaromatic hydrocarbons and polyaromatic heterocycles can be analyzed by ESI. In an ESI source, both radical cations and protonated molecules were formed and can be distinguished simultaneously for hydrocarbon compositions and nitrogen-containing components in highly aromatic condensed system based on accurate mass measurements. The results suggest that the applied ESI solvent composition has a significant impact on the detected species regarding the formation of either protonated molecules $[M + H]^+$ or radical cations ($M^{\bullet+}$) in the complex mixture where an electrochemical reaction (electron release of the analyte at the metal/solution interface) takes place inside the ion source. In our experiments, model compounds were selected in order to confirm our observations with heavy crude oil asphaltenes. Asphaltenes with the highly condensed nature and aromaticity are prime examples for the activation of radical cations because the ionization by electron transfer, such as the redox reaction, can occur easily in highly conjugated systems, such as polyaromatic hydrocarbons and polyaromatic heterocycles with lone pair electrons. In case of condensed aromatic structures, the number of the fused ring is determinative in the stability of radical cations because it has an influence on the level of delocalization of the lone electron and the positive charge in their π-electron system.[18]

As a consequence, our results suggest that the utility of electrospray ion source can be extended, and in addition to the polar components, the analysis of non-polar hydrocarbons can be successfully performed, especially in condensed aromatic structures such as asphaltenes.

2.6 References for Chapter 2

[1] E. C. Sanford. Interrelationship Between Oil Sand Fine Solids, Process Aids, Mechanical Energy and Oil Sand Age after Mining. *Can. J. Chem. Eng.* **1983**, *61*, 554-567.

[2] E. Y. Sheu. Petroleum Asphaltenes - Properties, Characterization, and Issues. *Energy Fuels* **2002**, *16*, 74-82.

[3] H. Groenzin, O. C. Mullins. Molecular Size and Structure of Asphaltenes from Various Sources. *Energy Fuels* **2000**, *14*, 677-684.

[4] O. C. Mullins, B. Martínez-Haya, A. G. Marshall. Contrasting Perspective on Asphaltene Molecular Weight. This Comment vs the Overview of A. A. Herod, K. D. Bartle, and R. Kandiyoti. *Energy Fuels* **2008**, *22*, 1765-1773.

[5] O. C. Mullins. The Modified Yen Model. *Energy Fuels* **2010**, *24*, 2179-2207.

[6] G. C. Klein, S. Kim, R. P. Rodgers, A. G. Marshall, A. Yen, S. Asomaning. Mass Spectral Analysis of Asphaltenes. I. Compositional Differences between Pressure-Drop and Solvent-Drop Asphaltenes Determined by Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2006**, *20*, 1965-1972.

[7] S. K. Panda, J. T. Andersson, W. Schrader. Characterization of Supercomplex Crude Oil Mixtures: What Is Really in There? *Angew. Chem. Int. Ed.* **2009**, *48*, 1788-1791.

[8] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Impact of Different Ionization Methods on the Molecular Assignments of Asphaltenes by FT-ICR Mass Spectrometry. *Anal. Chem.* **2012**, *84*, 5257-5267.

[9] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Characterization of Saturates, Aromatics, Resins, and Asphaltenes Heavy Crude Oil Fractions by Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2012**, *26*, 3481-3487.

[10] S. K. Panda, K.-J. Brockmann, T. Benter, W. Schrader. Atmospheric pressure laser ionization (APLI) coupled with Fourier transform ion cyclotron resonance mass spectrometry applied to petroleum samples analysis: comparison with electrospray ionization and atmospheric pressure photoionization methods. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 2317-2326.

[11] Y. Cho, J.-G. Na, N.-S. Nho, S. Kim, S. Kim. Application of Saturates, Aromatics, Resins, and Asphaltenes Crude Oil Fractionation for Detailed Chemical Characterization of Heavy Crude Oils by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Equipped with Atmospheric Pressure Photoionization. *Energy Fuels* **2012**, *26*, 2558-2565.

[12] K. E. Crawford, J. L. Campbell, M. N. Fiddler, P. Duan, K. Qian, M. L. Gorbaty, H. I. Kenttämaa. Laser-Induced Acoustic Desorption/Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for Petroleum Distillate Analysis. *Anal. Chem.* **2005**, *77*, 7916-7923.

[13] S. K. Panda, J. T. Andersson, W. Schrader. Mass-spectrometric analysis of complex volatile and nonvolatile crude oil components: a challenge. *Anal. Bioanal. Chem.* **2007**, *389*, 1329-1339.

[14] H. Müller, T. Andersson. Characterization of High-Molecular-Weight Sulfur-Containing Aromatics in Vacuum Residues Using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Anal. Chem.* **2005**, *77*, 2536-2543.

[15] S. Lababidi, S. K. Panda, J. T. Andersson, W. Schrader. Direct Coupling of Normal-Phase High-Performance Liquid Chromatography to Atmospheric Pressure Laser

Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for the Characterization of Crude Oil. *Anal. Chem.* **2013**, *85*, 9478-9485.

[16] J. B. Fenn, M. Mann, C. K. Meng, S. F. Wond, C. M. Whitehouse. Electrospray Ionization for Mass Spectrometry of Large Biomolecules. *Science* **1989**, *246*, 64-71.

[17] G. C. Klein, S. Kim, R. P. Rodgers, A. G. Marshall. Mass Spectral Analysis of Asphaltenes. II. Detailed Compositional Comparison of Asphaltenes Deposit to Its Crude Oil Counterpart for Two Geographically Different Crude Oils by ESI FT-ICR MS. *Energy Fuels* **2006**, *20*, 1973-1979.

[18] G. J. Berkel, S. A. McLuckey, G. L. Glish. Electrochemical Origin of Radical Cations Observed in Electrospray Ionization Mass Spectra. *Anal. Chem.* **1992**, *64*, 1586-1593.

[19] G. J. Berkel, K. G. Asano. Chemical Derivatization for Electrospray Ionization Mass Spectrometry. 2. Aromatic and Highly Conjugated Molecules. *Anal. Chem.* **1994**, *66*, 2096-2102.

[20] K. Miyabayashi, Y. Naito, K. Tsujimoto, M. Miyake. Structure characterization of polyaromatic hydrocarbons in Arabian mix vacuum residue by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Int. J. Mass spectrom.* **2004**, *235*, 49-57.

[21] H. Alboudwarej, J. Beck, W. Y. Svrcek, H. W. Yarranton, K. Akbarzadeh. Sensitivity of Asphaltene Properties to Separation Techniques. *Energy Fuels* **2002**, *16*, 462-469.

[22] M. E. Belov, R. Zhang, E. F. Strittmatter, D. C. Prior, K. Tang, R. D. Smith. Automated Gain Control and Internal Calibration with External Ion Accumulation Capillary Liquid Chromatography-Electrospray Ionization-Fourier Transform Ion Cyclotron Resonance. *Anal. Chem.* **2003**, *75*, 4195-4205.

[23] A. M. McKenna, L. J. Donald, J. E. Fitzsimmons, P. Juyal, V. Spicer, K. G. Standing, A. G. Marshall, R. P. Rodgers. Heavy Petroleum Composition. 3. Asphaltene Aggregation. *Energy Fuels* **2013**, *27*, 1246-1256.

[24] W. C. Hockaday, A. M. Grannas, S. Kim, P. G. Hatcher. Direct molecular evidence for the degradation and mobility of black carbon in soils from ultrahigh-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil. *Org. Geochem.* **2006**, *37*, 501-510.

[25] W. C. Hockaday, A. M. Grannas, S. Kim, P. G. Hatcher. The transformation and mobility of charcoal in a fire-impacted watershed. *Geochim. Cosmochim. Acta* **2007**, *71*, 3432-3445.

[26] A. J. Bard, L. R. Faulkner. Electrochemical methods: fundamentals and applications, John Wiley & Sons, Inc., New York, **1980**.

[27] R. G. Pearson, R. L. Dillon. Rates of Ionization of Pseudo Acids.1 IV. Relation between Rates and Equilibria. *J. Am. Chem. Soc.* **1953**, *75*, 2439-2443.

[28] P. Ballinger, F. A. Long. Acid Ionization Constants of Alcohols. II. Acidities of Some Substituted Methanols and Related Compounds. *J. Am. Chem. Soc.* **1960**, *82*, 795-798.

[29] G. J. Berkel, F. Zhou. Characterization of an Eiectrospray Ion Source as a Controlled-Current Electrolytic Cell. *Anal. Chem.* **1995**, *67*, 2916-2923.

[30] G. J. Berkel, F. Zhou. Electrospray as a Controlled-Current Electrolytic Cell: Electrochemical Ionization of Neutral Analytes for Detection by Electrospray Mass Spectrometry. *Anal. Chem.* **1995**, *67*, 3958-3964.

[31] M. G. Ikonomou, A. T. Blades, P. Kebarle. Electrospray-Ion Spray: A Comparison of Mechanisms and Performance. *Anal. Chem.* **1991**, *63*, 1989-1998.

[32] A. T. Blades, M. G. Ikonomou, P. Kebarle. Mechanism of Electrospray Mass Spectrometry. Electrospray as an Electrolysis Cell. *Anal. Chem.* **1991**, *63*, 2109-2114.
[33] G. J. V. Berkel, S. A. McLuckey, G. L. Glish. Preforming Ions in Solution via Charge-Transfer Complexation for Analysis by Electrospray Ionization Mass Spectrometry. *Anal. Chem.* **1991**, *63*, 2064-2068.
[34] Y. Kunihisa. Electrooxidation In Organic Chemistry: the Role of Cation Radicals As Synthetic Intermediates, Wiley, New York, **1984**.
[35] S. G. Lias, J. E. Bart, J. L. Holmes, R. D. Leven, J. F. Liebman, W. G. Mallard. Gas-Phase Ion and Neutral Thermochemistry. *J. Phys. Chem. Ref. Data* **1988**, *17*.
[36] W. Riepe, M. Zander. *Z. Naturforsch.* **1969**, *24a*, 2017.

CHAPTER 3.

ANALYSIS OF ASPHALTENES USING ONLINE COUPLING OF SIZE EXCLUSION CHROMATOGRAPHY AND ULTRAHIGH-RESOLUTION MASS SPECTROMETRY

Redrafted from "L. M. Guricza, W. Schrader. Analysis of asphaltenes using online coupling of size exclusion chromatography and ultrahigh-resolution mass spectrometry", will be submitted to Journal of Chromatography A.

3.1 Abstract

The asphaltene fraction is the heaviest part of a crude oil and is obtained as the fraction which contains compounds that are not soluble in paraffinic solvents such as *n*-heptane. Due to the limited solubility the use of separation methods is strongly reduced to methods that include solvents that dissolve asphaltenes. While ultrahigh-resolution mass spectrometry has been shown to be a method of choice for the detection of even asphaltene compounds, the separation of asphaltenes can only be accomplished by using separation methods such as size exclusion chromatography (SEC) which allows the use of the right solvents. Online coupling of size exclusion chromatography to mass spectrometry is a technique for gaining more detailed information about complex samples such as crude oil asphaltenes. Due to the separation the complexity of the asphaltene sample can be reduced, therefore mass spectra with increased information depth can be achieved due to the smaller amount of interacting species. Additionally, correlations can be proposed between molecular mass and structural characteristics of highly condensed aromatic molecules: compounds having both aromatic core and long aliphatic chains with higher masses were detected earlier and the smaller ones containing mostly highly aromatic structures and only a low amount of short alkyl chains (with the same DBE values) have more retention. Different experimental conditions such as stationary phase and mobile phase of asphaltene separation with size exclusion chromatography are investigated.

3.2 Introduction

The asphaltene fraction is the heaviest and most polar fraction of crude oil and is defined by their solubility in toluene and insolubility in normal paraffinic solvents (e.g. n-heptane).[1] After the separation procedure from a heavy crude oil[2] asphaltenes are still a heterogeneous mixture varying in both chemical composition and molecular size. The fraction consists of hydrocarbons and high amount of components with heteroatoms (N, O and S) and trace amount of metals (e.g. V, Ni, Fe). Structurally, asphaltene compounds are composed of highly condensed aromatic rings substituted with aliphatic chains.[1] The behavior of asphaltenes is highly influenced by the solvent and in some cases that can result in the formation of aggregates and flocculations, therefore the determination of their properties remains always a challenge.[3-5]

Heavy crude oils play an important role in replacing diminishing light oil resources. Therefore, the analysis of these resources having high asphaltene content with high viscosity and high heteroatom content, has drawn significant attention.[6] Asphaltene can cause blockages during oil production and during processing it can form coke deposits in reactors, additionally it can poison or deactivate catalysts.[6, 7] Therefore, there is a need to gain information on their characteristics, including their molecular weight and structure, which might help to understand these issues.

Several analytical techniques such as NMR or fluorescence measurements can give information about the bulk characteristics of asphaltenes, but these are average features describing only general characteristics.[8, 9] While the exploration of their elemental composition is established, there are still obscurities regarding the investigations of their molecular architecture. This is related to the exploration of molar mass range of asphaltenes present which have been debated in the literature.[5, 10-16]

Mass spectrometry (MS) with high mass accuracy and ultrahigh resolution can provide detailed information of molecular species even in extremely complex samples and has shown to be the method of choice for detailed petroleum analysis.[17] Information like unique elemental composition from accurate masses, component classes based on containing heteroatoms and level of aromaticity can be obtained easily.[8] Due to the high complexity matrix effects, ion discrimination suppression effects and the sensitivity of the instrument limit the type and amount of detected compounds.[8, 18, 19]

Thus, more detailed information can be obtained with coupling of different analytical methods. The combination of mass spectrometry and chromatographic separation is a possible way to reduce the complexity of the multiple samples and therefore increase the obtainable information depth.[20, 21] However, asphaltenes are not volatile enough for gas chromatographic analysis.[22, 23] Although, asphaltenes have a wide range of polarity, their aromatic feature and the van der Waals intermolecular attractions are more dominant.[1] Therefore, the difficulty of such an aromatic structure is their low solubility in solvents which are commonly used in reversed-phase liquid chromatography such as methanol and water. Finding an appropriate solvent system can be complicated therefore, strongly limiting the choice of chromatographic methods.

It is discussed that asphaltene molecules can differ in their molecular masses due to potential formation of associates.[3, 11] Taking into account the aforementioned conditions and limitations, size exclusion chromatography (SEC) seems to a very feasible method for the separation of asphaltenes. In SEC the molecules in solution are separated according to their hydrodynamic volume[24], so it is a suitable method for investigation of molecular mass distribution.[25, 26] For determination of the exact molecular size, polymer standards (e.g. polystyrene, poly(methyl methacrylate), polysaccharides) are usually required, although it is a critical point which can lead to ambiguous information.[27-29]

Direct coupling of SEC and MS has been reported to be a very promising technique in determination of molecular mass and to study e.g. polymeric products and aggregates.[30-32] The combination of one method that allows the separation according to molecular size in combination with the detection with ultrahigh resolution provides the opportunity of comprehensive evaluations on a molecular level. Due to the separation, lower amount of different species is present at the same time during each transient. This enables to reach less ion-ion interaction and avoid competition of different ions which result in higher sensitivity. Faster scanning is required to obtain as much information about the separation as possible without losing any separation information.[33] On the other hand the transient time should be long enough to have sensitive detection.[34] The difficulty of this approach is that an optimum needs to be found between the chromatographic separation and the transient length.

In this study different experimental conditions such as SEC columns with different pore sizes were investigated to find the optimized separation conditions. Furthermore,

different solvent systems involving tetrahydrofuran (THF), chloroform and toluene are compared as mobile phases in order to achieve the effective separation and the possibility of better characterization of highly aromatic compounds. In this respect, harmful and carcinogenic solvents such as benzene were aimed to be excluded.[35]

3.3 Experimental section

3.3.1 Sample preparation

Asphaltenes were precipitated from a heavy crude oil with n-heptane. using a modified IP 143 method.[36] 30 ml of n-heptane per g of heavy crude was mixed thoroughly. The mixture was then refluxed for 1 hour, then stored in the dark overnight. It was filtered through a Whatman filter paper (type 589.3) and the precipitated sample on the top of the filter paper was rinsed with hot n-heptane. It was then placed into a Soxhlet apparatus and extracted under reflux with n-heptane for additional 2 hours. The extraction was finalized by completely dissolve the asphaltenes from the filter paper with fresh toluene (ca. 20 hours). The toluene was evaporated under vacuum afterwards.

Chemicals used for sample preparation and for chromatographic separation are HPLC grade and were purchased from Sigma-Aldrich (Germany), except n-heptane (HPLC grade, Merck, Germany).

For SEC-MS measurements, the asphaltene sample (2 mg) was dissolved in 1 ml of solvent, THF and chloroform/toluene (70/30 and 30/70 v/v), respectively. For direct injection, further dilution was applied in order to reach the final concentration of 250 µg/ml.

3.3.2 Size exclusion chromatography

Size exclusion chromatography was performed by injecting 100 µl of sample using an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany). Five SEC columns were tested at room temperature, a Varian PLgel column (300 x 4.6 mm ID, 3 µm particle size, 10^2 Å pore size) and four PSS SDV (styrene-divinyl benzene copolymer) analytical columns (300 x 8.0 mm ID, 5 µm particle size) with different pore sizes: 10^2 Å, 10^3 Å, 10^5 Å and 10^6 Å, respectively. THF and mixtures of chloroform and

toluene (70/30 and 30/70 v/v) were used as mobile phases at a flow rate of 1 and 1.5 ml/min, respectively.

3.3.3 Mass spectrometry and data analysis

Mass analysis was performed on a research-type LTQ-Orbitrap Elite (Thermo Fisher, Bremen, Germany) which provides a maximum resolving power of up to 900 000 at *m/z* 400 equipped with a combination of APPI and APCI source. A Syagen Krypton VUV lamp (Syagen, Tustin, CA, USA) was used with photon emission at 10.0 and 10.6 eV. For online coupling of SEC-MS, the outlet of the UV detector was connected to the ionization source of the mass spectrometer using Teflon capillaries and the flow rate was adjusted with the help of a splitter. Thus, the sample was infused with a flow rate of 0.3 ml/min and evaporated in the heated nebulizer at 350 °C. Nitrogen was used as a continuous sheath gas flow and set to 20 (arbitrary unit). Samples were pumped through a transfer capillary (275 °C), the RF level of S-lenses was 60% and the discharge current for APCI needle was set to 4.0 μA. In case of direct infusion of the asphaltene samples, the same parameters were applied.

Data were collected in positive mode and processed with the LTQ FT Ultra 2.5.5 (Thermo Fischer Scientific, Bremen, Germany) data acquisition system with the mass range of *m/z* 200-1000 in full scan mode. The mass resolution was set to 240 000 (at *m/z* 400).

The acquired data were analyzed by Xcalibur 2.2 software (Thermo Fischer Scientific, Bremen, Germany) and Composer V1.5.0 software (Sierra Analytics, Modesto, CA, USA). The applied parameters and chemical constraints for the peak assignments were the followings: H: 0-1000, C: 0-200, N: 0-3, O: 0-3, S: 0-3, DBE: 0-40 with a maximum mass error of 1.4 ppm. The calculated molecular formulae were classified into different compound classes with a distinction of radical cations (X) and protonated ions (X[H]), as well. Excel (Microsoft Office Professional Plus 2010, Microsoft Excel 2010) and Origin (Origin 8.5.0) software were used for further data evaluation.

3.4 Results and discussion

3.4.1 Efficiency of separation

One of the major issues with mass spectrometric detection of complex samples is the difficulty to get a full representation of the sample due to suppression and sensitivity effects. An example is shown in Figure 3-1. Here, a mass spectrum from direct injection without any separation and two mass spectra (A and B) from different time ranges of online SEC-MS separation are compared. Due to the sample simplification, having less discrimination between the different species gives the possibility to detect new types of compounds such as NS[H], S[H], N_2S, O_3N[H] which formulas are highlighted in red. While e.g. at *m/z* of 482.19 in case of direct injection only $C_{37}H_{23}N$[H] is detected, in case of online SEC-MS additionally $C_{34}H_{27}NS$[H] can be assigned. Additionally, compounds such as $C_{32}H_{38}N_2S$ and a sulfur-containing isotope in its protonated form $C_{34}{}^{13}CH_{28}S$[H] could be detected in the selected mass range. Furthermore, N_3[H] is present with relatively higher intensity compared to the original asphaltene sample from direct injection. Sample simplification enables the sensitive detection of compounds being present only in small abundance.

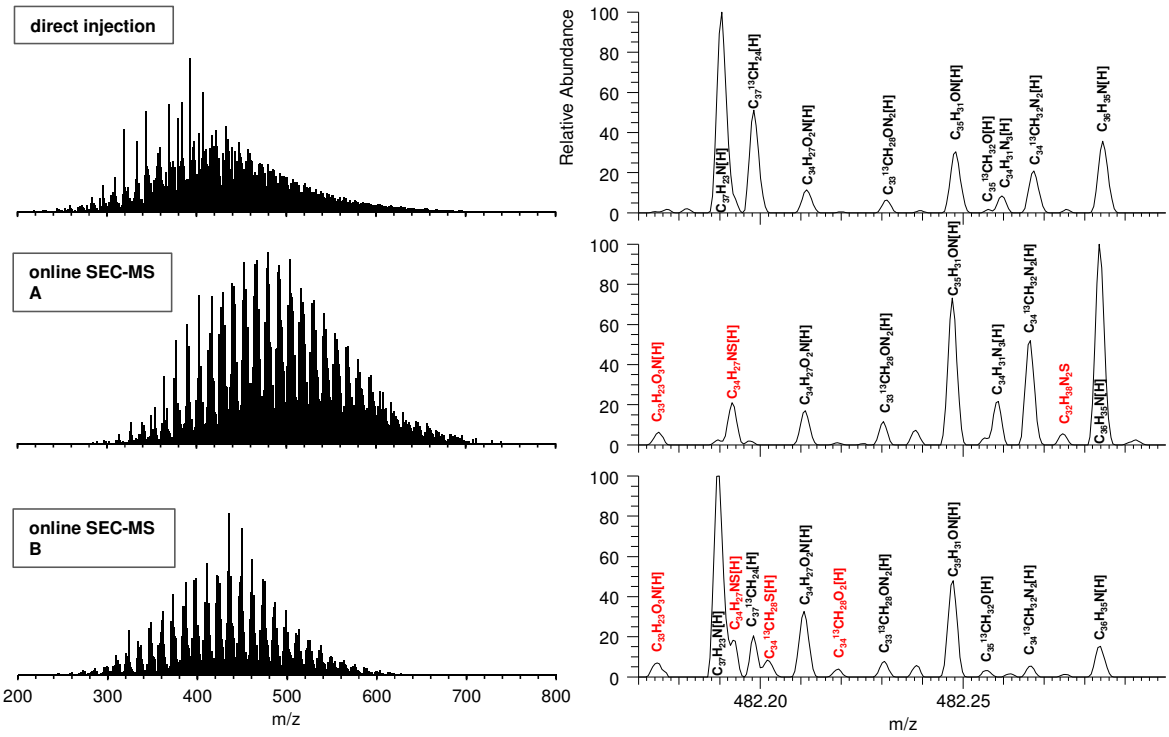

Figure 3-1. Mass spectra of asphaltenes with direct injection (top) and from different time ranges (A and B) of online SEC-MS coupling (center and bottom). Mass scale-expanded views of full mass spectra in the mass range of 482.17-482.30 Da (right column). Formulas, found only in case of online coupling SEC-MS results are highlighted with red font color.

3.4.2 Investigation of different columns and mobile phases

Different experimental conditions were investigated in order to accomplish the separation of asphaltenes by online SEC-MS. First of all, the selection of the column and stationary phase was optimized. In SEC the central issue is how well the analytes fit into the pores of the packing material inside the chromatographic column. Therefore, the pore size of the particles can be critical. Having not enough large pores means the total exclusion of some compounds which should be avoided. On the other hand, if the pore size is too large, that can cause long retention times and ruin the sensitivity by having too much diffusion. Furthermore, having the chromatographic peak as broad as possible during the separation helps increasing the amount of information that can be obtained about the sample. Therefore, the aim was to find an optimum for separation. Several columns were tested, a Varian PLgel column (300 x 4.6 mm 100 Å pore size) and four SDV analytical columns (300 x 8.0 mm, with pore sizes of 10^2 Å, 10^3 Å, 10^5 Å and 10^6 Å, respectively). On Figure 3-2 the total ion chromatograms (TIC) using different SDV column setups, only one column with pore size of 10^2 Å (**1**) and with 10^3 Å (**2**) or a combination of the two columns (**3**), are compared. A combination of columns with pore size of 10^3 and 10^2 Å was found to be the best choice, connected in series, because it accomplished the necessary and sufficient separation resulting in one broad chromatographic peak. Using only one column resulted in a peak width (measured at half maximum) of ca. 1.5-2 minutes, while in case of the combination of two columns it was ca. 4 minutes. Application of lower flow rate (1 ml/min instead of 1.5 ml/min) resulted in a broader peak with a peak width of more than 6 minutes (see Figure 3-3). The one broad chromatographic peak leads to the conjecture that no aggregation can be detected under the applied conditions when even scanning at higher mass range.[28]

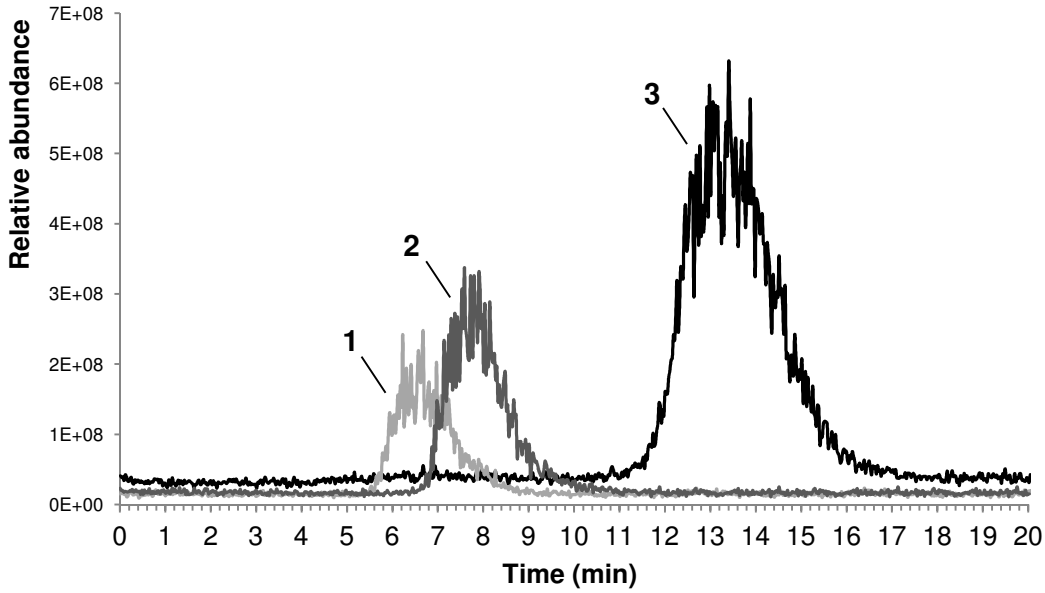

Figure 3-2. Total ion chromatogram (TIC) of asphaltenes in online SEC-MS coupling by using SDV columns with different pore sizes of particles inside the column as stationary phases (**1**: 10^2 Å pore size; **2**: 10^3 Å pore size, **3**: a combination of pores sizes of 10^3 Å and 10^2 Å, respectively).

With online coupling there is a possibility to do scan-to-scan evaluation. In this case with the applied settings (0.76 second transient) each second ca. 1.3 scans were recorded which means more than 450 individual mass spectra within the interesting time range (6 min). These can be calculated manually to get detailed information about the separation, although it is a little time-consuming. Instead, the combination of *1 minute* time ranges (approx. 75 scans) was applied to get a better overview about the separation. Figure 3-3 shows the TIC of asphaltenes with online SEC-MS coupling and the changes in *m/z* are represented at selected *1 minute* time ranges. The differences can be observed easily starting from *m/z* of 400-800 (**F1**) until that it reaches the *m/z* range of 200-600 (**F6**) (Figure 3-3, bottom left). Selection of even smaller time ranges (7-8 seconds, 9-10 scans) gives still tiny differences in regard of mass range, which is shown here with few examples of F2 fraction (Figure 3-3, bottom right, **F2/1**, **F2/2** and **F2/3**).

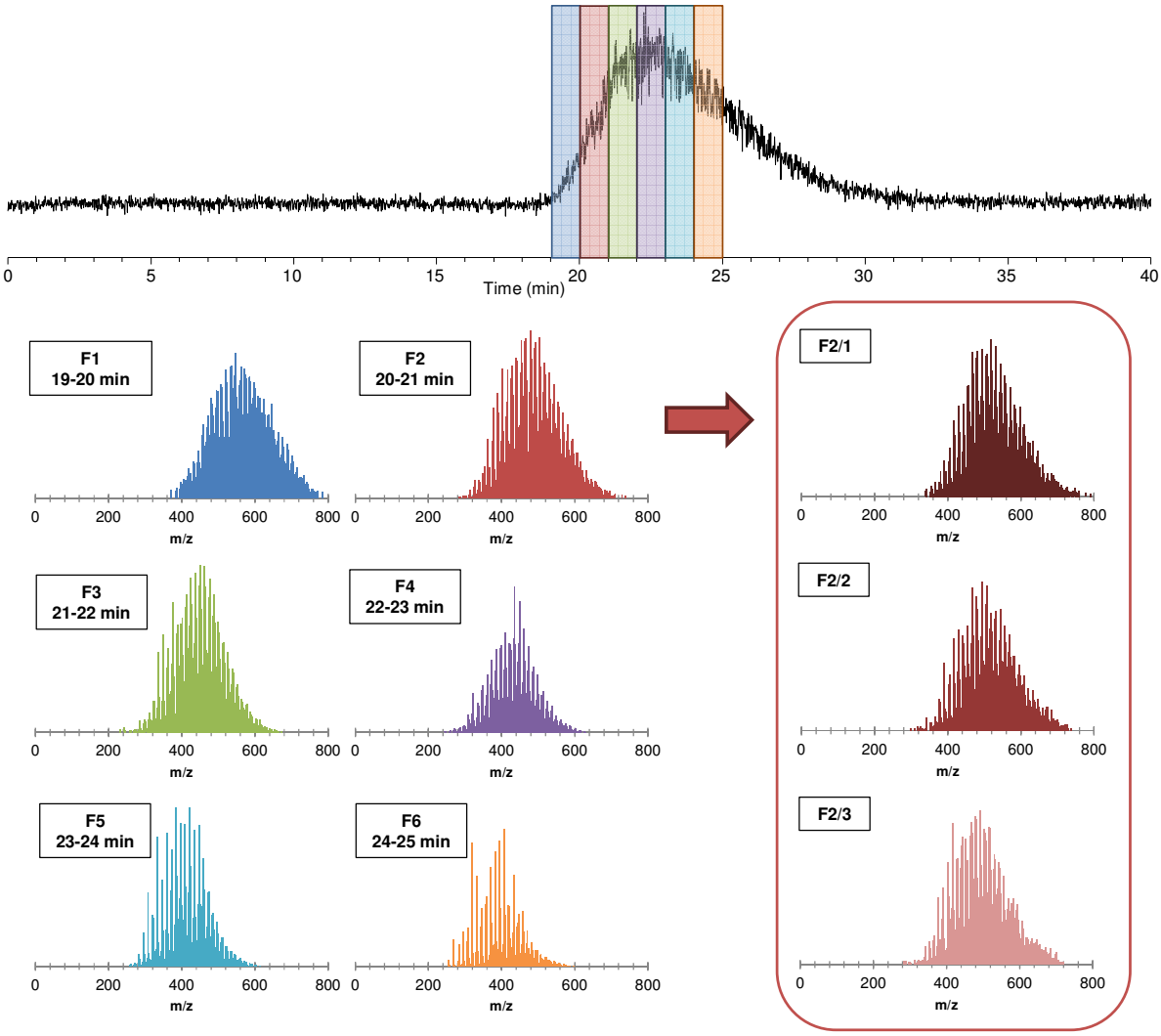

Figure 3-3. Total ion chromatogram (TIC) of asphaltenes with online SEC-MS coupling (top). Averaged mass spectra at selected time ranges (1 minute (bottom left) and 7-8 seconds (bottom right), respectively).

Size exclusion chromatography separates the compounds according to their hydrodynamic volume.[26] However, as we can see in Figure 3-3 this separation can be correlated to *m/z* values. As it is known, the detected species in crude oil are expected to be singly charged (z=1)[37], therefore here a molecular mass separation can be achieved. Compounds having higher masses are detected earlier and compounds with lower masses have longer retention times depending on the different exclusion and diffusion pathway of the molecules inside the pores of the stationary phase.

Different solvent systems such as pure THF and mixtures of chloroform and toluene were applied as mobile phases based on earlier studies[28] in order to evaluate the solvent effects in separation efficiency. The detected *m/z* values of the assigned compounds in each *1 minute* time range (F1-F6) were compared in case of pure THF,

and mixtures of chloroform and toluene (70/30 and 30/70 v/v). Figure 3-4 shows the Box plots of distributions of mass values through the SEC separation with the different solvent systems. Comparing for instance the *m/z* values of F1 time range, it is obvious that using THF resulted in the detection of data with the broadest mass range. Here, the median is at *m/z* 600, while using chloroform-toluene mixture the median is around *m/z* 500. As it can be seen in Figure 3-4, in case of THF the median decreases constantly along the separation until it reaches *m/z* ca. 400, while the decrease is not that significant for chloroform-toluene mixtures. Therefore, as a conclusion, pure THF provided the best separation in the selected time ranges (F1-F6).

Even though the separation is based on molecular masses, some interaction between the analytes and the column packing material as second retention mechanism can never be completely avoided.[29, 38] It has inevitably an influence on the performance of separation. This interaction can have an effect on the compounds at the later eluting part therefore the differences in masses are not that significant anymore (F4-F6) unlike as they are at the beginning of the separation (F1-F3). Smaller asphaltene molecules, as it can be suspected, may have stronger propensity to interact with the column packing material. Therefore, aromatic-aromatic (π-π) interactions can take place due to the similar aromatic structure between the analyte and the surface of stationary phase (styrene-divinyl benzene linkage).[39, 40] This interaction is more prominent where the aromatic feature of the compounds is more dominant and only a few aliphatic side chains are present. Solvents containing toluene might help in the establishment of aromatic-aromatic interaction and π-stacking therefore causing smearing during the separation. This can explain the slightly poorer separation performance when using a mobile phase with higher amount of toluene (Figure 3-4, bottom). This phenomenon is less pronounced with THF which might create a layer on the stationary phase preventing this pi interaction.

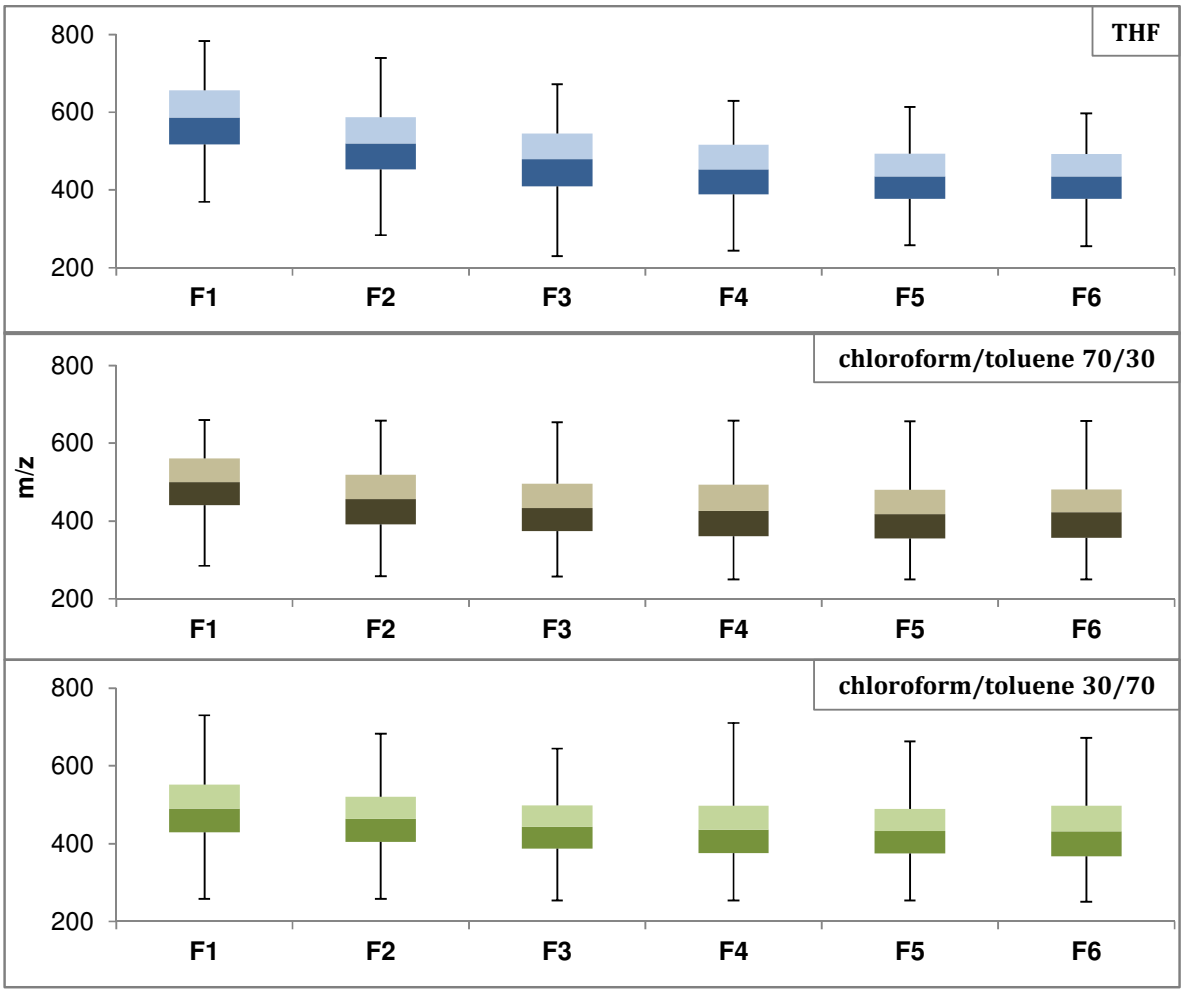

Figure 3-4. Box plots of distributions of m/z where the data from the mass spectra of 1 minute time ranges are summarized (F1-F6) through the SEC separation in cases of the different solvent systems: pure THF (top) and mixtures of chloroform and toluene (70/30 (center) and 30/70 v/v (bottom), respectively).

3.4.3 Investigation of separation using THF

Using THF as mobile phase results in good separation, therefore, this method was selected for the further investigation. The question of interest was to find some correlation between the molecular masses and the structural characteristics of the compounds. In order to investigate this, the central question was whether the separation occurs only according to molecular masses or also other properties are changing during the separation (e.g. compound classes, double bond equivalent (DBE)). The main assigned compound classes across the separation are shown in Figure 3-5. The intensity based diagram shows that there is only slight difference along the separation in compound classes (except O[H] compound class which intensity increases significantly). And based on the changes of the number of detected compounds, which is also not significant, there is no specific compound or group that dominates at a certain range of

the separation. Since ideally the separation is based only on the size without any chemical distinction, this was expected.

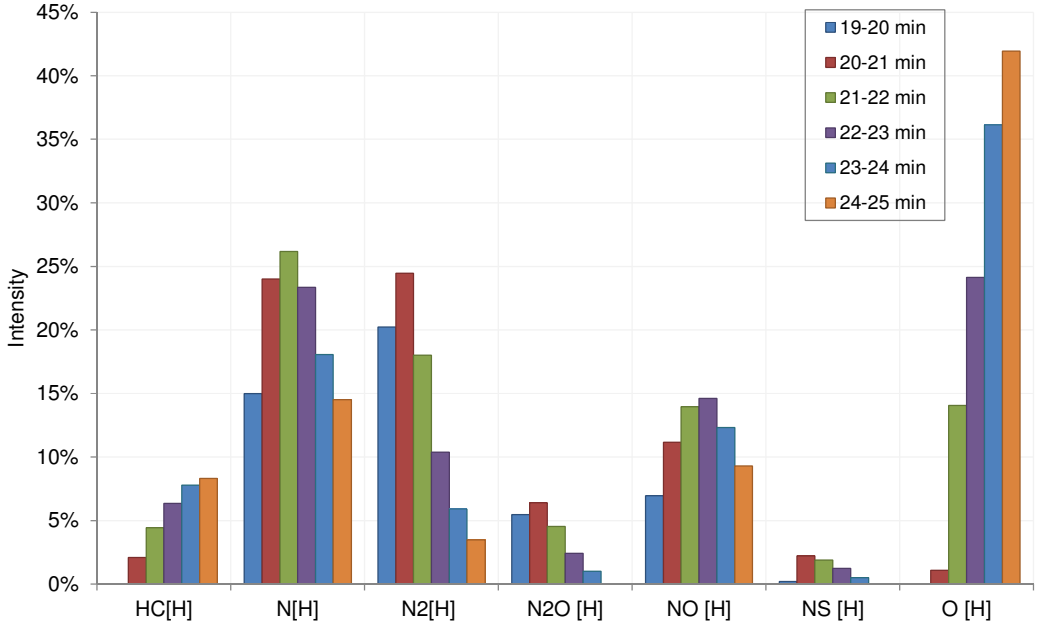

Figure 3-5. Intensity based class distribution of the main assigned compounds obtained by summarizing the mass spectra within 1 minute time ranges across the separation.

Furthermore, the average DBE numbers of all of the assigned compounds at selected ranges were calculated, as well (Figure 3-6). The results show that these compounds always have the same DBE range (DBE ranging in each case from 12 to 33) and median (22-23), independently from the retention time.

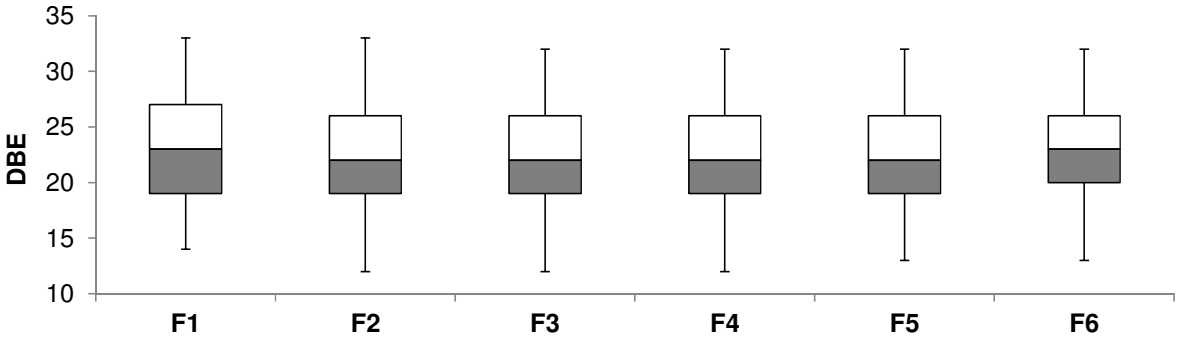

Figure 3-6. Box plots of distributions of DBE in cases of 1 minute time ranges (F1-F6) using pure THF mobile phase.

Having always the same DBE ranges but different masses during the separation confirms the assumption that large compounds having aromatic core together with long aliphatic side chains are eluting first (which are bigger) and compounds which are containing mostly condensed aromatic structures with only a few side chains with the similar DBE have larger retention (and are smaller). Therefore, the number of alkyl side

chains decreases along the separation which means that the overall H/C ratio decreases, as well. Since H/C is closely related to the aromaticity, the separation according to aromaticity is achieved in this process. Figure 3-7 illustrates the differences of H/C of all assigned compounds in cases of the two selected ranges (F2 and F5) (in the third axis it is scaled according to intensity).

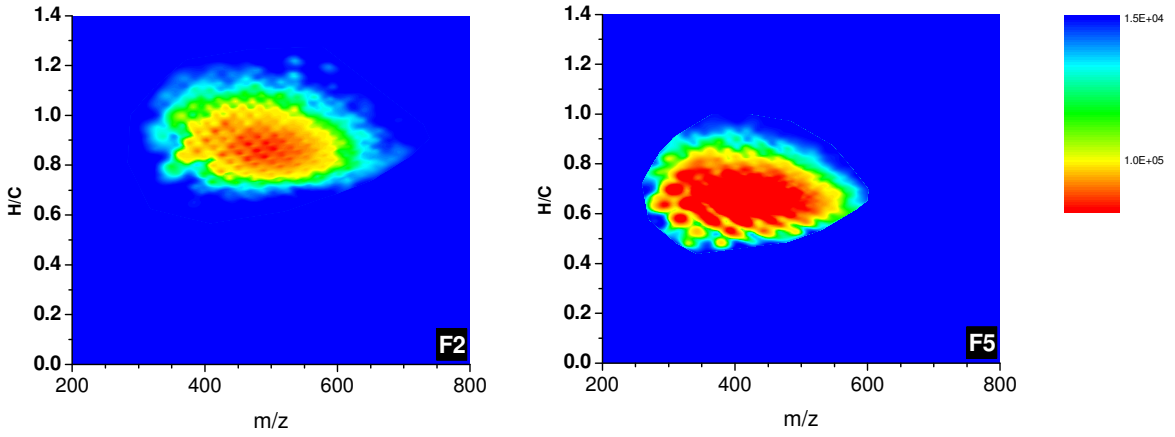

Figure 3-7. H/C distribution of all assigned compounds in cases of two selected time ranges (F2 and F5) of the SEC separation with THF (intensity is color-coded).

3.5 Conclusion

Asphaltenes as highly condensed polyaromatic compounds were investigated by using size exclusion chromatography in combination with ultrahigh-resolution mass spectrometry. According to mass spectrometric results, the masses of asphaltenes detected were below 1000 Da. Owing to the separation, reduced complexity could be achieved and more detailed information about the complex analyte could be gained compared to the direct injection. Thus, the separation made it possible to detect additional compound classes such as NS[H].

The results showed that the performance of separation depends significantly on the solvent system applied as mobile phase and the type of pore sizes of packing material inside the columns. Application of two columns connected in series resulted in a broad chromatographic peak. As the result of using THF as mobile phase, effective separation could be achieved not only according to *m/z* but also according to aromaticity, while the DBE range stayed constant along the separation. Therefore, compounds having the same aromatic core but are different in length of their side alkyl chains could be detected separately.

3.6 References for Chapter 3

[1] O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall. Asphaltenes, Heavy Oils, and Petroleomics, Springer, New York, **2007**.

[2] B. N. Barman, V. L. Cebolla, L. Membrado. Chromatographic Techniques for Petroleum and Related Products. *Crit. Rev. Anal. Chem.* **2000**, *30*, 75-120.

[3] O. C. Mullins. The Modified Yen Model. *Energy Fuels* **2010**, *24*, 2179-2207.

[4] T. N. Loegel, N. D. Danielson, D. J. Borton, M. R. Hurt, H. I. Kenttämaa. Separation of Asphaltenes by Reversed-Phase Liquid Chromatography with Fraction Characterization. *Energy Fuels* **2012**, *26*, 2850-2857.

[5] E. Y. Sheu. Petroleum AsphaltenesProperties, Characterization, and Issues. *Energy Fuels* **2002**, *16*, 74-82.

[6] O. C. Mullins. Review of the Molecular Structure and Aggregation of Asphaltenes and Petroleomics. *SPE-95801-PA* **2008**, *13*, 48-57.

[7] R. B. de Boer, K. Leerlooyer, M. R. P. Eigner, A. R. D. van Bergen. Screening of Crude Oils for Asphalt Precipitation: Theory, Practice, and the Selection of Inhibitors. *SPE-24987-PA* **1995**, *10*, 55-61.

[8] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Impact of Different Ionization Methods on the Molecular Assignments of Asphaltenes by FT-ICR Mass Spectrometry. *Anal. Chem.* **2012**, *84*, 5257-5267.

[9] O. C. Mullins. The Asphaltenes. *Annu. Rev. Anal. Chem.* **2011**, *4*, 393-418.

[10] F. Trejo, J. Ancheyta, T. J. Morgan, A. A. Herod, R. Kandiyoti. Characterization of Asphaltenes from Hydrotreated Products by SEC, LDMS, MALDI, NMR, and XRD. *Energy Fuels* **2007**, *21*, 2121-2128.

[11] A. A. Herod, K. D. Bartle, R. Kandiyoti. Characterization of Heavy Hydrocarbons by Chromatographic and Mass Spectrometric Methods: An Overview. *Energy Fuels* **2007**, *21*, 2176-2203.

[12] O. C. Mullins, B. Martínez-Haya, A. G. Marshall. Contrasting Perspective on Asphaltene Molecular Weight. This Comment vs the Overview of A. A. Herod, K. D. Bartle, and R. Kandiyoti. *Energy Fuels* **2008**, *22*, 1765-1773.

[13] A. A. Herod, K. D. Bartle, R. Kandiyoti. Comment on a Paper by Mullins, Martinez-Haya, and Marshall "Contrasting Perspective on Asphaltene Molecular Weight. This Comment vs the Overview of A. A. Herod, K. D. Bartle, and R. Kandiyoti". *Energy Fuels* **2008**, *22*, 4312-4317.

[14] S. Badre, C. C. Goncalves, K. Norinaga, G. Gustavson, O. C. Mullins. Molecular size and weight of asphaltene and asphaltene solubility fractions from coals, crude oils and bitumen. *Fuel* **2006**, *85*, 1-11.

[15] A. R. Hortal, P. Hurtado, B. Martínez-Haya, O. C. Mullins. Molecular-Weight Distributions of Coal and Petroleum Asphaltenes from Laser Desorption/Ionization Experiments. *Energy Fuels* **2007**, *21*, 2863-2868.

[16] R. C. Shea, S. C. Habicht, W. E. Vaughn, H. I. Kenttämaa. Design and Characterization of a High-Power Laser-Induced Acoustic Desorption Probe Coupled with a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. *Anal. Chem.* **2007**, *79*, 2688-2694.

[17] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Characterization of Saturates, Aromatics, Resins, and Asphaltenes Heavy Crude Oil Fractions by Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2012**, *26*, 3481-3487.

[18] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah. Mechanistic investigation of ionization suppression in electrospray ionization. *J. Am. Soc. Mass. Spectrom.* **2000**, *11*, 942-950.

[19] H. Trufelli, P. Palma, G. Famiglini, A. Cappiello. An overview of matrix effects in liquid chromatography–mass spectrometry. *Mass Spectrom. Rev.* **2011**, *30*, 491-509.

[20] P. Arpino. Combined liquid chromatography mass spectrometry. Part I. Coupling by means of a moving belt interface. *Mass Spectrom. Rev.* **1989**, *8*, 35-55.

[21] A. Makarov, M. Scigelova. Coupling liquid chromatography to Orbitrap mass spectrometry. *J. Chromatogr. A* **2010**, *1217*, 3938-3945.

[22] B. James W, C. Donald E, in *Chemistry of Asphaltenes, Vol. 195*, American Chemical Society, **1982**, pp. 219-236.

[23] W. Schrader, S. K. Panda, J. T. Andersson. Mass-spectrometric analysis of complex volatile and nonvolatile crude oil components: a challenge. *Anal. Bioanal. Chem.* **2007**, *389*, 1329-1339.

[24] G. H. Lathe, C. R. J. Ruthven. The separation of substances and estimation of their relative molecular sizes by the use of columns of starch in water. *Biochem. J* **1956**, *62*, 665-674.

[25] E. S. P. Bouvier, S. M. Koza. Advances in size-exclusion separations of proteins and polymers by UHPLC. *TrAC, Trends Anal. Chem.* **2014**, *63*, 85-94.

[26] A. M. Striegel, W. W. Yau, J. J. Kirkland, D. D. Bly. Modern Size Exclusion Chromatography, Practice of Gel Permeation and Gel Filtration Chromatography, 2nd ed., John Wiley & Sons., Hoboken, New Jersey, **2009**.

[27] F. Karaca, C. A. Islas, M. Millan, M. Behrouzi, T. J. Morgan, A. A. Herod, R. Kandiyoti. The Calibration of Size Exclusion Chromatography Columns: Molecular Mass Distributions of Heavy Hydrocarbon Liquids. *Energy Fuels* **2004**, *18*, 778-788.

[28] S. Dong, A. M. Striegel. Size-Exclusion Chromatography of Asphaltenes: An Experimental Comparison of Commonly Used Approaches. *Chromatographia* **2013**, *76*, 725-733.

[29] S. Sato, T. Takanohashi. Molecular Weight Calibration of Asphaltenes Using Gel Permeation Chromatography/Mass Spectrometry. *Energy Fuels* **2005**, *19*, 1991-1994.

[30] L. Prokai, W. J. Simonsick. Electrospray ionization mass spectrometry coupled with size-exclusion chromatography. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 853-856.

[31] T. Reemtsma, A. These. On-line Coupling of Size Exclusion Chromatography with Electrospray Ionization-Tandem Mass Spectrometry for the Analysis of Aquatic Fulvic and Humic Acids. *Anal. Chem.* **2003**, *75*, 1500-1507.

[32] A. Ah Toy, P. Vana, T. P. Davis, C. Barner-Kowollik. Reversible Addition Fragmentation Chain Transfer (RAFT) Polymerization of Methyl Acrylate: Detailed Structural Investigation via Coupled Size Exclusion Chromatography–Electrospray Ionization Mass Spectrometry (SEC–ESI-MS). *Macromolecules* **2004**, *37*, 744-751.

[33] S. Lababidi, S. K. Panda, J. T. Andersson, W. Schrader. Direct Coupling of Normal-Phase High-Performance Liquid Chromatography to Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for the Characterization of Crude Oil. *Anal. Chem.* **2013**, *85*, 9478-9485.

[34] W. Schrader, H.-W. Klein. Liquid chromatography/Fourier transform ion cyclotron resonance mass spectrometry (LC-FTICR MS): an early overview. *Anal. Bioanal. Chem.* **2004**, *379*, 1013-1024.

[35] T. Ghislain, P. Faure, R. Michels. Detection and Monitoring of PAH and Oxy-PAHs by High Resolution Mass Spectrometry: Comparison of ESI, APCI and APPI Source Detection. *J. Am. Soc. Mass. Spectrom.* **2012**, *23*, 530-536.

[36] H. Alboudwarej, J. Beck, W. Y. Svrcek, H. W. Yarranton, K. Akbarzadeh. Sensitivity of Asphaltene Properties to Separation Techniques. *Energy Fuels* **2002**, *16*, 462-469.

[37] C. A. Hughey, R. P. Rodgers, A. G. Marshall. Resolution of 11 000 Compositionally Distinct Components in a Single Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrum of Crude Oil. *Anal. Chem.* **2002**, *74*, 4145-4149.

[38] E. Al-Muhareb, T. J. Morgan, A. A. Herod, R. Kandiyoti. Characterization of Petroleum Asphaltenes by Size Exclusion Chromatography, UV-fluorescence and Mass Spectrometry. *Pet. Sci. Technol.* **2007**, *25*, 81-91.

[39] C. A. Hunter, J. K. M. Sanders. The nature of .pi.-.pi. interactions. *J. Am. Chem. Soc.* **1990**, *112*, 5525-5534.

[40] M. L. Waters. Aromatic interactions in model systems. *Curr. Opin. Chem. Biol.* **2002**, *6*, 736-741.

CHAPTER 4.

ARGENTATION CHROMATOGRAPHY COUPLED TO ULTRAHIGH-RESOLUTION MASS SPECTROMETRY FOR EFFECTIVE SEPARATION OF A HEAVY CRUDE OIL

Redrafted from "L. M. Guricza, W. Schrader. Argentation chromatography coupled to ultrahigh-resolution mass spectrometry for effective separation of a heavy crude oil", submitted to Journal of Chromatography A.

4.1 Abstract

Simplification of highly complex mixtures such as crude oil by using chromatographic methods makes it possible to get more detailed information about the elaborate analyte. Separation by argentation chromatography can be achieved based on the different interaction between the silver ions (Ag^+) immobilized on the silica gel surface and the π-bonds of the analytes. A heavy crude oil containing compounds with high amount of heteroatoms (N, O, S) and high degree of unsaturation was analyzed by online coupling of argentation chromatography and ultrahigh-resolution mass spectrometry. This setup gives an additional dimension for sorting the compounds according to their double bond equivalents (DBE) which correlates to their aromaticity, and allows sensitive detection on a molecular level. In case of heavy crude oil presented in this study compounds possessing DBE with 20-25 analyzed with direct injection while after separation unsaturated compounds with DBE up to 30-35 could be detected. The reduced complexity achieved by the separation helps increasing the information depth.

4.2 Introduction

The energy demand is very high in this modern community and according to predictions the need for energy is growing in the coming years. Studying the effectiveness of different energy sources and making improvements is one of the main issues nowadays in order to resolve concerns about the future energy supplies.

Among the possible resources, fossil fuel still draws a special interest because it can produce a significant amount of energy per unit mass and its chemical compounds are storing a large amount of energy. However, due to the tendency of diminishing conventional crude oil, more viscous heavy crude oils (e.g. from tar sands and oil shale) are becoming more important.[1] Understanding the behavior of crude oil components on a molecular level is essential to improve the existing upgrading technologies and methods. In case of heavy crude oil there is a need of investigating their chemical and structural composition because it is known that due to their high content of heteroatoms (N, O, S) difficulties (e.g. precipitation, catalyst poisoning, gum formation) can occur during their treatment.[2] Their characterization has always been a challenge especially due to their high complexity.

Ultrahigh-resolution mass spectrometry is a method of choice for detection of such complex systems to get information on a molecular level. However, using this analytical tool alone is not sufficient. Discrimination and suppression effects of certain compounds may occur which can be an obstacle for full characterization due to the large amounts of ions present at the same time.[3-5] Additionally, mass data only show elemental composition but do not allow differentiating structural differences of isomeric compounds within a given formula.

In order to overcome this problem and reduce the complexity, additional separation has to be applied. Several chromatographic methods have been studied (e.g. NP-HPLC, SEC) on crude oil based on different types of interactions and mechanisms between the various surface of stationary phase and the complex analyte.[6-11]

Since heavy crude oil compounds contain a large number of unsaturation and condensed aromatic and heteroaromatic rings, argentation chromatography (also known as silver ion chromatography) can provide a selective separation tool for the separation of crude oils where interactions between double bonds and silver(I) ions on the stationary phase provide reversible complex formation with different strength.

Argentation chromatography was first applied in the early 1960s for separation of lipid structures.[12] Since then it and has been successfully used on investigation of complex fatty acid mixtures [13-15], on isolation of natural products [16] and studies on food chemistry [17-19]. The principle behind the separation, which was first described by Winstein and Lucas in 1938, is the weak interaction occurring between the silver ions (Ag^+) and compounds containing π-bonds.[20] The mechanism of complex formation of silver(I) ions (possessing empty s- and p-orbitals) with ligands having π-system has been discussed in the literature in detail.[21-23] The original suggestion involves only the deformation of π-orbitals of the olefins, which could be described by four different resonance structures.[20] Another assumption, according to Dewar and co-workers [24] is that the bonding in the complex formation involves both a σ-bond - between the highest occupied molecular orbital (HOMO) of an olefin and the unoccupied s-orbital of silver(I) - and a π-bond - between the lowest unoccupied molecular orbital (LUMO) of an olefin and a filled d-orbital of silver(I) - depending on the availability of electrons due to steric factors and electrostatic effects.[23, 25] This second alternative seems to be more commonly accepted.[12]

The main limitation of the application of argentation chromatography has been the stability and the mobility of silver(I) ions. Recent applications and investigations show that in case of silver ion immobilization by covalent bonds onto the thiol moiety of mercaptopropyl modified silica gel (see Figure 4-1), silver ions retain well during leaching test and they are stable upon light exposure.[26, 27] It results in an effective separation of polycyclic aromatic hydrocarbons (compounds with maximum four or five aromatic rings) by the number of rings [27, 28] and mixtures of alkenes having different number of double bonds [26].

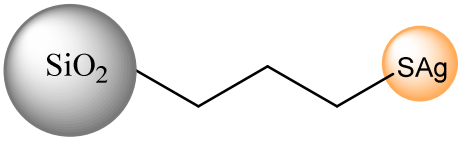

Figure 4-1. Silver(I)-mercaptopropyl silica gel.

We have shown that this concept is working for the difficult to dissolve asphaltene fraction of crude oil [29], the use of argentation chromatography on a whole crude oil

necessitates the change of the mobile phase system while it also increases the complexity of the analyte sample making the separation even more difficult because the column can easily being overloaded. Here, the direct coupling of argentation chromatography with an ultrahigh-resolution Orbitrap mass spectrometer is shown studying a heavy crude oil containing compounds having up to 7-8 condensed aromatic rings. The major difficulty of this method arises from the high complexity of the sample, thus the present functional groups and heteroatoms are increasing the number and the type of interactions which can occur between the analyte molecules and the stationary phase.[27] Using online coupling of ultrahigh-resolution mass spectrometry for detection makes possible to get fast and detailed information for the eluted compounds across the chromatographic separation and allows following the changes in detail.

4.3 Experimental section

4.3.1 Sample preparation

Chemicals used for sample preparation, for synthesis and for chromatographic separation are HPLC grade and were purchased from Sigma-Aldrich (Germany) if it is not stated otherwise.

For measurements, a heavy crude oil sample of North American source (3-6 mg) was dissolved in 1 ml of a mixture of toluene and chloroform (7:3 v/v). For direct injection, further dilution was applied in order to reach the final concentration of 250 µg/ml.

4.3.2 Argentation chromatography

The stationary phase (silver(I)-mercaptopropyl silica gel) is not commercially available, therefore it was synthesized in our laboratory using the procedure reported by Nocun et al.[28] Two different silica gels were used as starting materials with a particle size of 10 µm (LiChrosorb Si 100 (Merck, Darmstadt, Germany)) and 5 µm (Polygosil 100 (Macherey-Nagel, Düren, Germany)), respectively. The silica gel was dried at 200 °C for 24 h to remove adsorbed water. 10 g of silica gel was suspended in 50 ml dry toluene and in 15 ml 3-mercaptopropanotrimetoxysilane and it was stirred for 24 h at 120 °C under argon atmosphere. Toluene used in the syntheses was dried over 3 Å molecular sieves. The prepared 3-mercaptopropyl functionalized silica gel was filtered and washed with dry toluene and methanol. The resulting solid material was

dried at 50 °C overnight then it was stirred in 250 ml of silver nitrate aqueous solution (0.1 M) for 3 h at room temperature. The obtained silver(I)-mercaptopropyl silica gel was filtered, washed with water and methanol then dried at 50 °C overnight. This modified silica gel was then loaded into a stainless steel column (250 mm x 4.6 mm and 250 mm x 2.0 mm, respectively) with the help of Knauer pneumatic pump at 400 bar using isopropanol (IPA) as packing solvent. The column was then equilibrated for further chromatographic measurements with a mixture of cyclohexane (Acros Organics, Geel, Belgium) and dichloromethane.

Argentation chromatography was performed on an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Bremen, Germany) by injecting 100 µl and 20 µl of sample, respectively. A mixture of cyclohexane and chloroform (7:3 v/v) was used as mobile phase with increasing amount of isopropanol up to 10% (v/v) at a flow rate of 2 ml/min and 0.3 ml/min, respectively. The purpose of using isopropanol was to compete with the analyte compounds and supersede them from the coordination with the silver(I) ions of the stationary phase. The separation of the molecules was based on the strength of their interaction. The amount of IPA was increased stepwise during the whole separation process: after every 12 min it was set to the following percentage: 0.5%; 2%; 5% and 10%, respectively.

4.3.3 Mass spectrometry and data analysis

Mass analysis was performed on a research-type LTQ-Orbitrap Elite (Thermo Fisher Scientific, Bremen, Germany) which provides a resolving power of up to 900 000 at *m/z* 400. Atmospheric pressure photoionization (APPI) was used for ionization to detect compounds with a wide polarity range.[30, 31] For APPI a Krypton VUV lamp (Syagen Technologies, Tustin, CA, USA) was applied with photon emission at 10.0 and 10.6 eV.

For online coupling LC/MS measurement the outlet of the UV detector was directly connected to the ionization source of the mass spectrometer using PEEK capillaries. The sample was infused at a flow rate of 0.3 ml/min using a splitter. The heated nebulizer was operated at 350 °C and nitrogen was used as sheath gas.

Data were collected in positive mode in a mass range of *m/z* 200-800 using full scan mode and processed with the LTQ FT Ultra 2.5.5 (Thermo Fischer Scientific, Bremen, Germany). Mass resolution (R) was set to 120 000 and 480 000 (at *m/z* 400), respectively.

Xcalibur 2.2 software (Thermo Fischer Scientific, Bremen, Germany) and Composer V1.5.0 software (Sierra Analytics, Modesto, CA, USA) were used for extended data analysis. For calculations and peak assignments the following constraints regarding the number of possible elements were applied: H: 0-100, C: 0-50, N: 0-3, O: 0-3, S: 0-3, and double bond equivalent (DBE): 0-40. The calculated molecular formulae were classified into different heteroatom classes with a distinction of radical cations (M) and protonated ions (M[H]), as well. Excel (Microsoft Office Professional Plus 2010, Microsoft Excel 2010) software was used for further data evaluation and graphical representation.

During evaluation DBE is used as an important parameter for comparison of the different components. DBE refers to the sum of the rings and the number of double bonds within a molecule and is calculated from the molecular formula ($C_cH_hN_nO_oS_s$) of the assigned individual peaks by the following equation: $DBE = c - \frac{h}{2} + \frac{n}{2} + 1$.

4.4 Results and discussion

4.4.1 Argentation chromatography

In order to study the interaction between the silver ions (Ag^+) and the unsaturated compounds in a heavy crude oil, a modified silica gel stationary phase had to be synthesized and utilized on a heavy crude oil mixture based on a study reported for the separation of polycyclic aromatic sulfur heterocycles.[28] The application here was extended into studying not only sulfur-containing compounds but diverse types of compound classes with different heteroatoms (N, O, S) present in the sample and additionally do this online with direct detection by an ultrahigh-resolving mass spectrometer. An overview of the optimized separation is shown in Figure 4-2 which shows the UV chromatogram recorded at 254 nm and the corresponding stepwise increased amount of IPA.

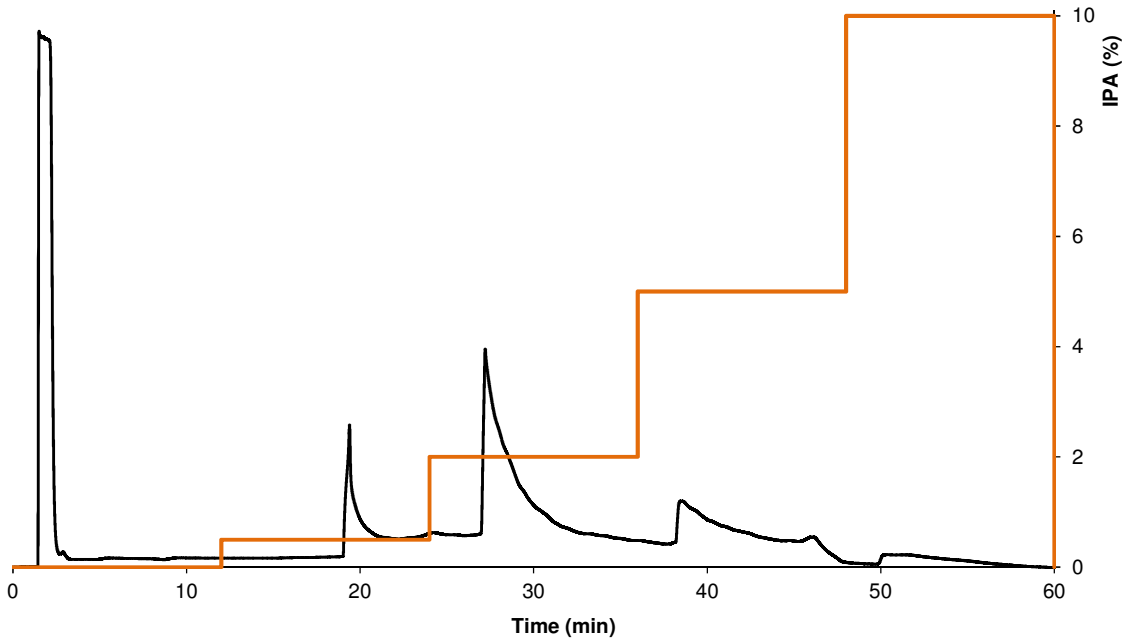

Figure 4-2. UV chromatogram at λ=254 nm and the corresponding amount of IPA of argentation chromatographic separation of heavy crude oil on a HPLC column (250 mm x 4.6 mm, 10 µm).

The strength of the possible interactions between the silver(I) ions and the analyte is weak enough to be broken by the addition of a few percent of so-called competitive ligand into the mobile phase. In this case IPA is applied to supersede the coordinated unsaturated compounds of crude oil. Starting with a mixture of cyclohexane and chloroform (7:3 v/v) the amount of IPA was increased stepwise four times (0.5%, 2%, 5% and 10%) after each 12 min in order to investigate the effect of this additional solvent and to evaluate the different fractions at each step individually. The effect of this stepwise increase of IPA amount can be seen in Figure 4-2, where the retention of compounds is recorded around 20, 28, 39 and 50 min, respectively.

Due to the high complexity and variability of the sample no one can expect sharp peaks and compound selective separation of a crude oil. The crude oil with an estimated number of more than one million different chemical components [32] is just way too complex to achieve this goal. The analyte consists of different heteroatom classes that have the same chemical composition but different structures leading to interactions with diverse strength between the analyte and the stationary phase.[10] Depending on the length of the different alkyl side chains of the analyte, the strength of the interaction can vary, as well. Although, this effect is ideally not expected to play a significant role, but based on other studies, alkyl chains with different length can have different effects

on the retention.[10, 28] Compounds having longer alkyl side chains elute earlier compared to the shorter alkyl chain possibly due to the less interaction with the stationary phase. This effect can be especially pronounced when the complexity of the highly unsaturated sample is a very critical factor.

4.4.2 Investigation of the separation efficiency

One of the most important advantages of this separation is that the complexity of the sample is reduced based on the selected type of interaction. In this case the separation is primarily based on the degree of unsaturation. The efficiency of this separation can be demonstrated by selecting different time ranges of the separation meaning different amount of competitive solvent (IPA) and comparing the mass spectra in these mass windows. Figure 4-3 illustrates the averaged full mass spectra and an expanded mass scale using 0% IPA in the time range of 0-12 min (top), 2% IPA in the time range of 24-36 min (middle) and 10% IPA in the time range of 48-60 min. Following the differences along the separation within the individual steps is also possible by the calculation of each individual scans owing to the online coupling, but in this case it did not result in fine separation due to the high complexity of the sample. Here, the focus was on the effect of stepwise increased IPA. Therefore, averaged mass spectra are displayed with the most important assignments. As the example shows, a compound with the formula $C_{42}H_{68}$ having DBE of 9 (detected as $[C_{42}H_{69}]^+$ protonated ion) can be eluted at the beginning of the separation without using a competitive ligand within the range of *m/z* 573.2-573.6 which is shown on the mass scale-expanded views (Figure 4-3, right column, top). After adding 2% IPA this compound is not present. Instead, within the same mass range, for example hydrocarbon compounds with elemental composition of $C_{43}H_{56}$ having DBE of 16 and $C_{44}H_{44}$ having DBE of 23 can be detected in their protonated form (see in Figure 4-3 as $[C_{43}H_{57}]^+$ and $[C_{44}H_{45}]^+$, respectively) which possess higher unsaturation. Moreover, using 10% of IPA even more unsaturated compounds can be detected, a hydrocarbon with DBE of 30 $[C_{45}H_{33}]^+$ being an example. The same or similar trends can be observed in case of the sulfur- and the oxygen-containing compounds. When increasing the IPA content, the most intensive signals within a nominal mass are shifting towards the lower mass defects, which correspond to higher unsaturation and more condensed structures. [33, 34]

Nitrogen-containing compounds are another interesting issue. They are not detected at all in the early retention times without IPA. However, with sufficient amount of IPA more and more N-species can be observed and at the highest IPA content (10%) a large amount of N-compounds are present with fairly high DBE (more than 30). (See results and discussions below.)

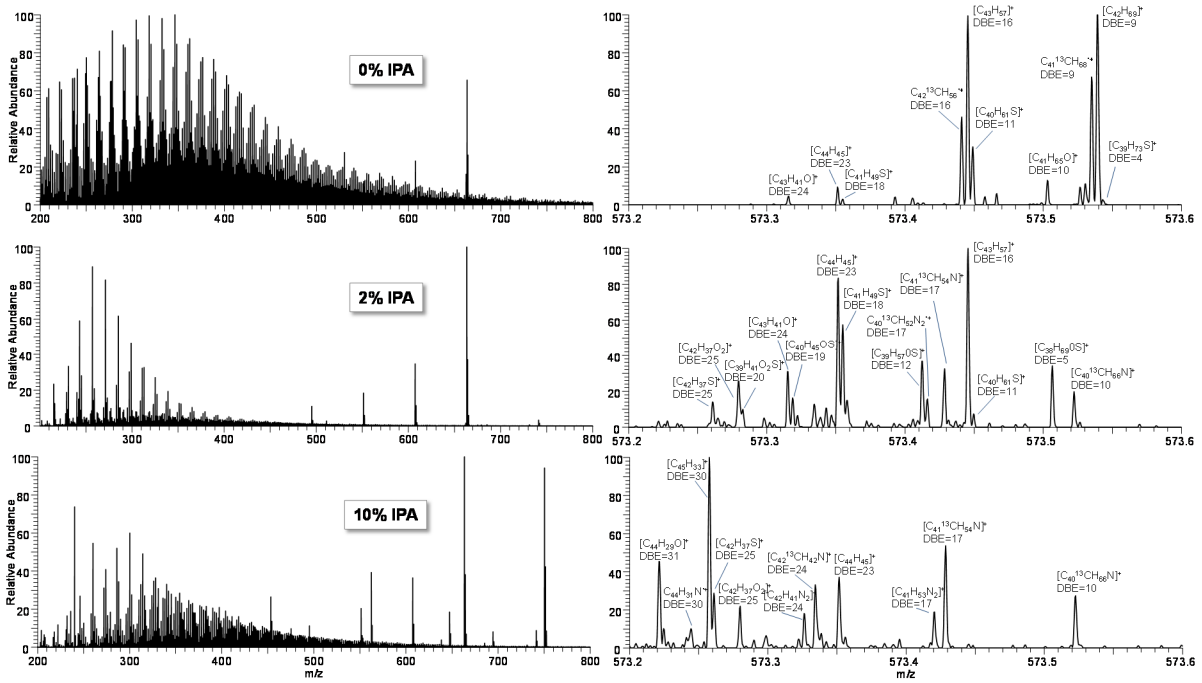

Figure 4-3. Averaged mass spectra of heavy crude oil obtained during online LC/MS separation using cyclohexane/chloroform (7:3 v/v) as mobile phase with 0% IPA (left, top), 2% IPA (left, middle) and 10% IPA (left, bottom). The corresponding mass scale-expanded views of full mass spectra with the most important assigned peaks in the mass range of m/z 573.2-573.6 (right column).

Overall, the complexity is reduced at the different stages leading to reduced ion suppression and discrimination effects, and increased effectiveness of the separation allowing an investigation on a molecular level by mass spectrometry.

4.4.3 Ultrahigh-resolution mass spectrometry

Online coupling makes the easy and fast optimization of the different parameters possible which are playing a role during the separation (e.g. the necessary amount of IPA for elution of certain compounds and compound classes). Additionally, it gives the opportunity to optimize the conditions for a possible fraction collection procedure. However, finding an optimum for transient time and resolution is always a challenge e.g. in order to have sufficiently high sensitivity without overloading a column.[35] The resolution should be high enough to be able to differentiate the peaks for components

which masses with small mass splits but low enough (which means short transients) in order not to lose too much chromatographic separation. The importance of ultrahigh resolution in this case is shown in Figure 4-4. The obtained mass spectra in the mass range of 387.15-387.35 are compared in case of resolving power of 120 000 (top) and 480 000 (bottom) at *m/z* 400. The need for sufficiently high resolution is due to overlapping signals in such complex mixtures, especially with polyaromatic hydrocarbons and polyaromatic sulfur heterocycles present, which can cause wrong peak assignment. The mass difference between the protonated hydrocarbon (e.g. $[C_{29}H_{39}]^+$ at *m/z* of 387.30463) and the corresponding protonated sulfur-containing compound ($[C_{26}H_{43}S]^+$ at *m/z* of 387.30800) is 3.4 mmu, one of the major mass splits. In case of R=120 000 the above mentioned ions are not detected separately and the result is a fairly wide overlapping peak with an assignment for either the first or the second chemical composition with higher error, while the application of R=480 000 can provide the signals for both species with assignments with low errors. Therefore having a resolution of 480 000 is necessary in this case.

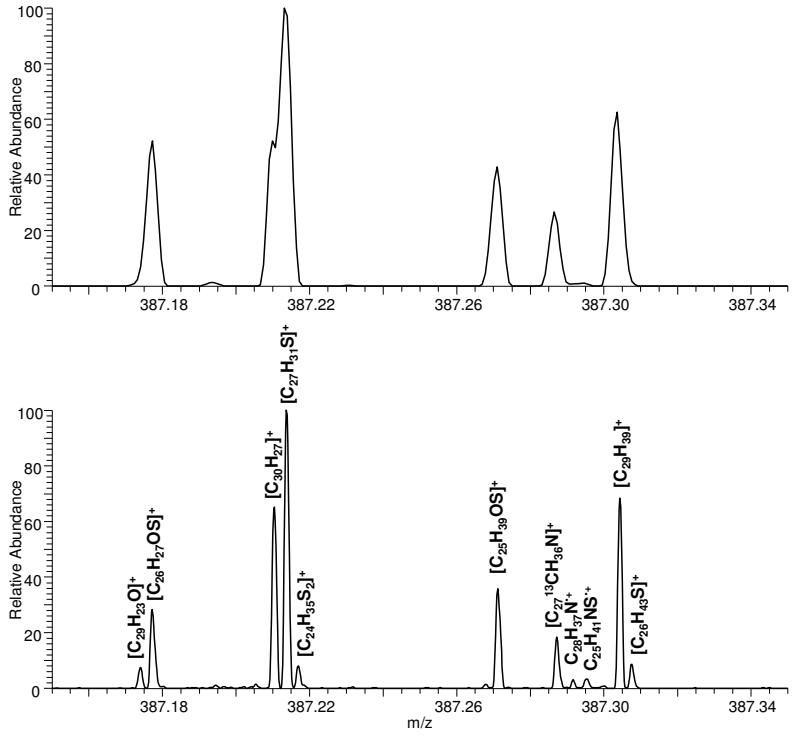

Figure 4-4. Mass scale-expanded views of full mass spectra of heavy crude oil with resolution of 120 000 (top) and 480 000 (at m/z 400) (bottom) obtained by cyclohexane/chloroform (7:3 v/v) containing 2% of IPA.

4.4.4 Evaluation of the heteroatom classes

Due to the high complexity of heavy crude oil the evaluation of the efficiency and interpretation of the separation can be difficult. Besides the interaction between the silver(I) ion and π-bonds, the coordination of the possible heteroatoms (N, O, S) and the affinity towards the silver(I) ions might contribute to the strength of the complex formation.[36, 37] Therefore compounds with heteroatoms spend more time on the surface of the stationary phase and have longer retention times compared to the hydrocarbons. In this regard, the evaluation according to DBE values (according to unsaturation) of individual heteroatom classes was selected for further characterizing the separation. Since the abundance of hydrocarbons, N_1-, O_1- and S_1-containing species is very high in this sample, these types of compound classes are evaluated with more details. In general, increasing the amount of competitive solvent (IPA) resulted in increasing ranges of DBE distribution at each individual steps. The importance of the characteristic of the stationary phase and the column is remarkable here. To study the differences between using silver(I) column on 10 µm material and on 5 µm material the stationary phase was synthesized and two different columns were prepared. As a comparison, the difference between the mass spectra recorded in the mass range of *m/z* 250-550 using 10% IPA in case of the two columns is shown in Figure 4-5. Silver(I) modified silica gel with 5 µm particle size and 250 mm x 2.0 mm column resulted in sharper chromatographic peak shapes and more intensive peaks in mass spectra compared to the one with 10 µm size (filled into 250 mm x 4.6 mm column). This presumably is due to the fact that in the first case the dead volume is smaller (because of the geometry) and on the other hand the flow rate is ca. 7-times lower. Therefore the dilution of the sample is reduced which results in higher sensitivity. This makes it possible to detect important compounds with high DBE by mass spectrometry which are not easily ionized and/or are suppressed in case of using the 250 mm x 4.6 mm, 10 µm column. Additional results are shown which were recorded using the 250 mm x 2.0 mm, 5 µm column, because here we were able to perform much better peak assignments and calculations.

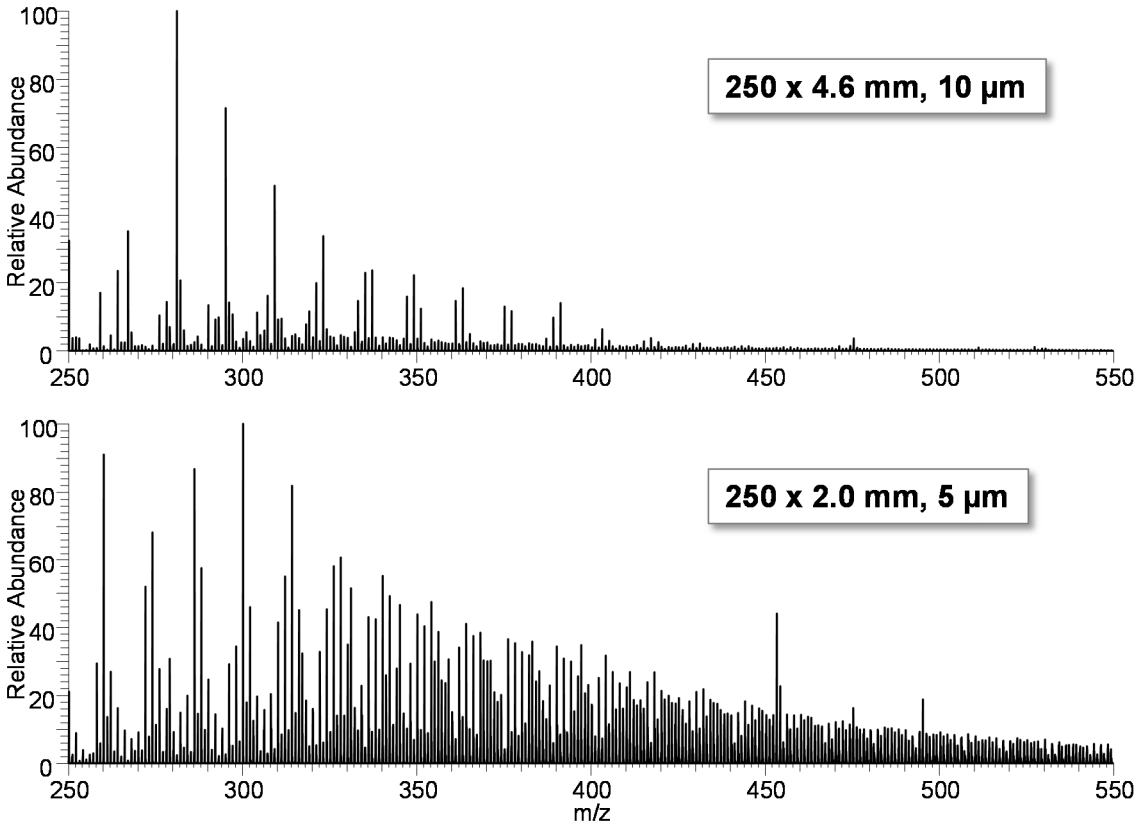

Figure 4-5. Mass spectra recorded during the separation using 10% IPA in the mass range of m/z 250-550 with 250 x 4.6 mm, 10 µm column (up) and with 250 x 2.0 mm, 5 µm column (bottom).

Kendrick plots [33] provide a meaningful representation about selected properties of the different types of assigned compounds in the complex crude oil sample after calculation of each individual signal in mass spectrum. The Kendrick plot (DBE against carbon number) for hydrocarbons is compared in Figure 4-6 between direct injection and separation, where the different hues of blue color represent the assigned hydrocarbons (both protonated and radicals) at the different steps along the separation. The DBE was in the range of 3-15 without using IPA, 5-20 with 0.5% IPA, 10-25 with 2% IPA, 13-30 with 5% IPA and 15-35 with 10% IPA. It is apparent that these results can embrace a large DBE range and much higher DBE range can be covered compared to the direct injection where the detected DBE range was between 3 and 28. This phenomenon can be observed not only for hydrocarbons but also for other heteroatom classes.

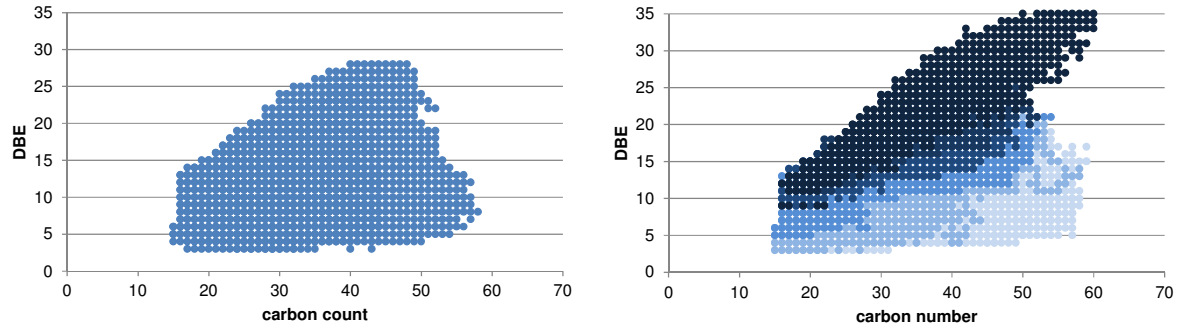

Figure 4-6. Kendrick plot (DBE plotted against carbon number) for hydrocarbons (M and M[H]) in case of direct injection (left) and along the separation where the different hues of blue color represent the assigned hydrocarbons at the different steps along the separation using argentation chromatographic separation of heavy crude oil on a HPLC column (250 mm x 2.0 mm, 5 µm). It has to be noted that [H] describes a protonated molecule while the absence of it describes a radical ion.

In Figure 4-7 the DBE distribution of assigned hydrocarbons, N_1-, O_1- and S_1-containing compounds are shown. Here the focus is on the comparison of the corresponding assigned species (radical and protonated) between the direct injection and specifically the last step of the separation (using 10% IPA). In case of each heteroatom class a selectively high DBE range (up to 30-35) can be detected with high intensity when 10% IPA is applied. It is 15-30 for hydrocarbons, 5-30 for N_1 compounds, 20-35 for O_1 compounds and 15-33 for S_1 compounds.

Another obvious observation for all classes shown in Figure 4-7 with the exemption of the N_1 class, is that for the fraction with 10% IPA only a small band of compounds are separated that are close to the planar limit boundary.[38] Here, compounds with high aromaticity are separated with only small alkyl side chains. This shows that the removal of such highly aromatic compounds from the stationary phase needs a higher concentration of the competitive ligand.

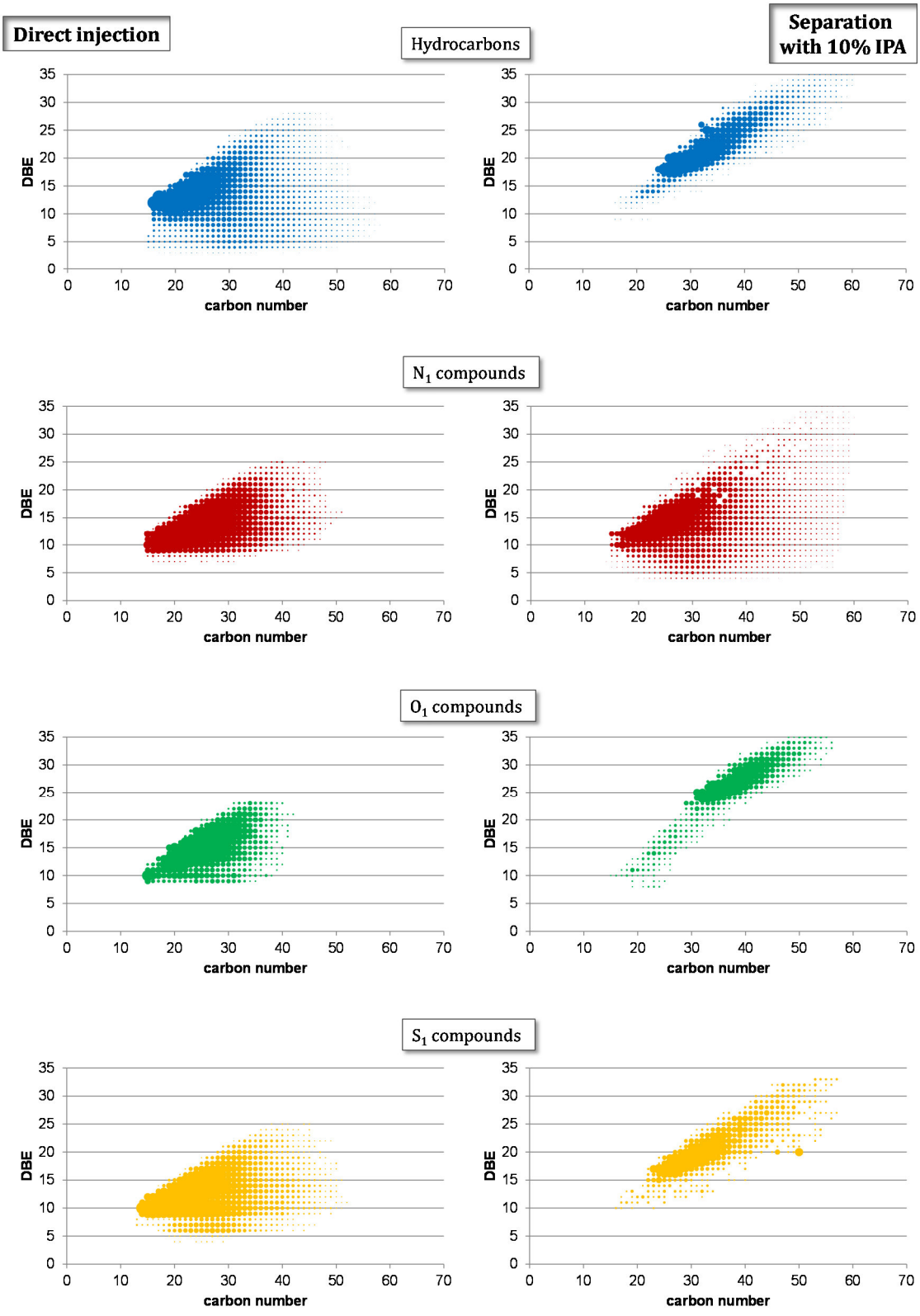

Figure 4-7. A comparison of the direct injection and the last step of the separation (using 10% IPA) in case of assigned hydrocarbons, N_1-, O_1- and S_1-containing compounds (M and M[H]). (The intensity is related to the size of the bubbles.)

For N_1 species this range is not narrow, but it is prominent that compounds with a higher DBE than 25 are present although they are detected with small intensity. The

behavior of nitrogen compounds on the column is therefore different to hydrocarbons and other heterocycles. Due to the higher polarity the complexation behavior with the Ag^+-ions is stronger, resulting in longer retention on the stationary phase and only a higher concentration of the competitive ligand allows washing those compounds from the column.

Overall, this enables selective separation of asphaltene-type compounds with high aromaticity having up to 10-12 fused aromatic rings.[38]

4.5 Conclusion

The efficiency of self-synthesized silver(I)-mercaptopropyl silica gel stationary phase (with 10 and 5 µm particle size) is studied by the investigation of heavy crude oil using online coupling of argentation chromatography and ultrahigh-resolution mass spectrometry. Although, the separation of the highly complex mixture has only one dimension (additional pre-separation e.g. SARA fractionation [39] methods are not included), due to the direct coupling and ultrahigh resolution it is possible to achieve fast and detailed data analysis on a molecular level and selectively highlight compounds within specific DBE ranges. Here, the utilization of using 5 µm particle sized material for the separation is providing a big improvement on the separation. This phase is selective towards the number of π-bonds within the molecules; furthermore it is influenced by the presence of heteroatoms and alkyl groups.

Depending on the amount of IPA as competitive solvent in the mobile phase, compounds with increasing number of DBE can be detected along the separation. With this setup, compounds containing only few double bonds can be easily separated from the ones with DBE of 30-35 within a complex crude oil mixture. This study shows that heterocyclic compounds having up to 10-12 aromatic rings with and without the presence of heteroatoms (N, O, S) at the same time can be highlighted by the presented online LC/MS coupling.

4.6 References for Chapter 4

[1] S. Chopra, L. Lines. Special section: Heavy Oil. *The Leading Edge* **2008**, *27*.

[2] O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall. Asphaltenes, Heavy Oils, and Petroleomics, Springer, New York, **2007**.

[3] S. K. Panda, J. T. Andersson, W. Schrader. Characterization of Supercomplex Crude Oil Mixtures: What Is Really in There? *Angew. Chem. Int. Ed.* **2009**, *48*, 1788-1791.

[4] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Impact of Different Ionization Methods on the Molecular Assignments of Asphaltenes by FT-ICR Mass Spectrometry. *Anal. Chem.* **2012**, *84*, 5257-5267.

[5] P. Schmitt-Kopplin, M. Englmann, R. Rossello-Mora, R. Schiewek, K. J. Brockmann, T. Benter, O. J. Schmitz. Combining chip-ESI with APLI (cESILI) as a multimode source for analysis of complex mixtures with ultrahigh-resolution mass spectrometry. *Anal. Bioanal. Chem.* **2008**, *391*, 2803-2809.

[6] K. Sripada, J. Andersson. Liquid chromatographic properties of aromatic sulfur heterocycles on a Pd(II)-containing stationary phase for petroleum analysis. *Anal. Bioanal. Chem.* **2005**, *382*, 735-741.

[7] C. S. Hsu, K. Qian. High-boiling aromatic hydrocarbons characterized by liquid chromatography-thermospray-mass spectrometry. *Energy Fuels* **1993**, *7*, 268-272.

[8] K. J. Leontaritis, G. A. Mansoori. Fast crude-oil heavy-component characterization using combination of ASTM, HPLC, and GPC methods. *J. Pet. Sci. Technol.* **1989**, *2*, 1-12.

[9] A. A. Herod, K. D. Bartle, R. Kandiyoti. Characterization of Heavy Hydrocarbons by Chromatographic and Mass Spectrometric Methods: An Overview. *Energy Fuels* **2007**, *21*, 2176-2203.

[10] S. K. Panda, W. Schrader, J. T. Andersson. β-Cyclodextrin as a stationary phase for the group separation of polycyclic aromatic compounds in normal-phase liquid chromatography. *J. Chromatogr. A* **2006**, *1122*, 88-96.

[11] S. A. Wise, S. N. Chesler, H. S. Hertz, L. R. Hilpert, W. E. May. Chemically-bonded aminosilane stationary phase for the high-performance liquid chromatographic separation of polynuclear aromatic compounds. *Anal. Chem.* **1977**, *49*, 2306-2310.

[12] L. J. Morris. Separations of lipids by silver ion chromatography. *J. Lipid Res.* **1966**, *7*, 717-732.

[13] W. W. Christie, E. Y. Brechany, K. Stefanov. Silver ion high-performance liquid chromatography and gas chromatography—mass spectrometry in the analysis of complex fatty acid mixtures: application to marine invertebrates. *Chem. Phys. Lipids* **1988**, *46*, 127-135.

[14] B. Nikolova-Damyanova, B. G. Herslof, W. W. Christie. Silver ion high-performance liquid chromatography of derivatives of isomeric fatty acids. *J. Chromatogr. A* **1992**, *609*, 133-140.

[15] R. O. Adlof. Normal-phase separation effects with lipids on a silver ion high-performance liquid chromatography column. *J. Chromatogr. A* **1997**, *764*, 337-340.

[16] S. B. Mathur, L. Gonzalez. Identification of Terpenoids From the Leaves of Piptocarpha paradoxa and Their Biological Activities. *J. Nat. Prod.* **1982**, *45*, 495-496.

[17] P. H. Laakso, K. V. V. Nurmela, D. R. Homer. Composition of the triacylglycerols of butterfat and its fractions obtained by an industrial melt crystallization process. *J. Agric. Food Chem.* **1992**, *40*, 2472-2482.
[18] K. Chakraborty, R. P. Raj. Eicosapentaenoic Acid Enrichment from Sardine Oil by Argentation Chromatography. *J. Agric. Food Chem.* **2007**, *55*, 7586-7595.
[19] J. K. Kramer, C. Cruz-Hernandez, Z. Deng, J. Zhou, G. Jahreis, M. E. Dugan. Analysis of conjugated linoleic acid and trans 18:1 isomers in synthetic and animal products. *Am. J. Clin. Nutr.* **2004**, *79*, 1137S-1145S.
[20] S. Winstein, H. J. Lucas. The Coordination of Silver Ion with Unsaturated Compounds. *J. Am. Chem. Soc.* **1938**, *60*, 836-847.
[21] H. J. Lucas, R. S. Moore, D. Pressman. The Coordination of Silver Ion with Unsaturated Compounds. II. cis- and trans-2-Pentene1. *J. Am. Chem. Soc.* **1943**, *65*, 227-229.
[22] L. J. Andrews, R. M. Keefer. Cation Complexes of Compounds Containing Carbon--Carbon Double Bonds. IV. The Argentation of Aromatic Hydrocarbons. *J. Am. Chem. Soc.* **1949**, *71*, 3644-3647.
[23] J. G. Traynham, M. F. Sehnert. Ring Size and Reactivity of Cyclic Olefins: Complexation with Aqueous Silver Ion1,2. *J. Am. Chem. Soc.* **1956**, *78*, 4024-4027.
[24] J. S. Dewar. A review of the pi-complex theory. *Bull. Soc. Chim. Fr.* **1951**, *18*, C71-C79.
[25] J. G. Traynham, J. R. Olechowski. Complexation of Cyclic Olefins with Aqueous Silver Ion and with Molecular Iodine1,2. *J. Am. Chem. Soc.* **1959**, *81*, 571-574.
[26] J. C. Aponte, J. T. Dillon, R. Tarozo, Y. Huang. Separation of unsaturated organic compounds using silver–thiolate chromatographic material. *J. Chromatogr. A* **2012**, *1240*, 83-89.
[27] J. A. Dunn, K. B. Holland, J. R. Jezorek. Argentation liquid chromatography of polynuclear aromatic hydrocarbons on a silver(l)-loaded mercaptopropyl silica gel stationary phase. *J. Chromatogr. A* **1987**, *394*, 375-381.
[28] M. Nocun, J. T. Andersson. Argentation chromatography for the separation of polycyclic aromatic compounds according to ring number. *J. Chromatogr. A* **2012**, *1219*, 47-53.
[29] L. M. Guricza, W. Schrader. New Separation Approach for Asphaltene Investigation: Argentation Chromatography Coupled with Ultrahigh-Resolution Mass Spectrometry. *Energy Fuels* **2015**, *29*, 6224-6230.
[30] J. M. Purcell, C. L. Hendrickson, R. P. Rodgers, A. G. Marshall. Atmospheric Pressure Photoionization Proton Transfer for Complex Organic Mixtures Investigated by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 1682-1689.
[31] L. C. Short, S.-S. Cai, J. A. Syage. APPI-MS: Effects of mobile phases and VUV lamps on the detection of PAH compounds. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 589-599.
[32] J. Beens, J. Blomberg, P. J. Schoenmakers. Proper Tuning of Comprehensive Two-Dimensional Gas Chromatography (GC×GC) to Optimize the Separation of Complex Oil Fractions. *J. High Resolut. Chromatogr.* **2000**, *23*, 182-188.
[33] E. Kendrick. A Mass Scale Based on CH2 = 14.0000 for High Resolution Mass Spectrometry of Organic Compounds. *Anal. Chem.* **1963**, *35*, 2146-2154.
[34] C. Ovalles, C. E. R. Jr. Analytical Methods in Petroleum Upstream Applications, 1st edition ed., CRC Press 2015, **2015**.
[35] S. Lababidi, S. K. Panda, J. T. Andersson, W. Schrader. Direct Coupling of Normal-Phase High-Performance Liquid Chromatography to Atmospheric Pressure Laser

Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for the Characterization of Crude Oil. *Anal. Chem.* **2013**, *85*, 9478-9485.

[36] R. Zhou, S. Z. Qiao. Silver/Nitrogen-Doped Graphene Interaction and Its Effect on Electrocatalytic Oxygen Reduction. *Chem. Mater.* **2014**, *26*, 5868-5873.

[37] A. V. Novikov, R. A. Bublyaev, N. V. Krasnov, Y. P. Kozmin, O. A. Mirgorodskaya. ESI-MS studies of silver ion competitive interaction with cysteine-containing peptides and sulfur-containing amino acids. *Protein Pept Lett.* **2010**, *17*, 1392-1397.

[38] Y. Cho, Y. H. Kim, S. Kim. Planar Limit-Assisted Structural Interpretation of Saturates/Aromatics/Resins/Asphaltenes Fractionated Crude Oil Compounds Observed by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Anal. Chem.* **2011**, *83*, 6068-6073.

[39] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Characterization of Saturates, Aromatics, Resins, and Asphaltenes Heavy Crude Oil Fractions by Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2012**, *26*, 3481-3487.

CHAPTER 5.

NEW SEPARATION APPROACH FOR ASPHALTENE INVESTIGATION: ARGENTATION CHROMATOGRAPHY COUPLED WITH ULTRAHIGH-RESOLUTION MASS SPECTROMETRY

Redrafted from "L. M. Guricza, W. Schrader. New separation approach for asphaltene investigation: argentation chromatography coupled with ultrahigh-resolution mass spectrometry. *Energy Fuels* **2015**, 29, 6224-6230" Copyright 2015 American Chemical Society (ACS).

5.1 Abstract

Argentation chromatography has been developed for the analysis and separation of asphaltenes. The separation is based on the interaction between the silver ions (Ag^+) bonded to a silica gel surface and the π-systems of the polycondensed aromatic compounds, where the interactions can be different when heteroatoms (N, O, S) are present in the molecules. Online coupling of argentation chromatography and high-resolution mass spectrometry allows fast and detailed analysis of the separation of crude oil asphaltenes. Highly condensed aromatic compounds possessing a high degree of unsaturation can be successfully separated using a mixture of toluene and chloroform (7:3 v/v) as mobile phase and dimethyl sulfoxide as competitive ligand for elution of asphaltene compounds with double bond equivalents (DBE) of up to 40. Making use of the different structural features of the components, it is possible to separate isomeric compounds that interact with different strengths with the stationary phase. The composition of mobile phase plays a significant role in separation efficiency.

5.2 Introduction

Heavy crude oil resources have attracted enhanced interest over the past few years in the oil industry owing to increased energy needs and the diminishing availability of light oils.[1] As a result of the extreme complexity [2] and high aromaticity of the bulk material of heavy crude oil and other unfavorable features (e.g., poor solubility, aggregation propensity, high viscosity), problems can occur during transportation, separation, and refining processes, such as catalyst poisoning, fouling, and precipitation.[3] In heavy crude oil, compounds with condensed aromatic structures, with or without heteroatoms present, have a proclivity to precipitate inside the pipeline on the walls and/or at the pipe junctions, causing problems such as clogging. These problems are mostly associated with the asphaltene fraction of the crude oil. Based on solubility, using the so-called SARA fractionation, crude oil can be separated into different fractions.[4] In this procedure, the initial step is the removal of asphaltenes using a non-polar solvent, while the remaining fraction called maltenes are further separated into saturates, aromatics, and resins according to their solubility.

The asphaltene fraction is defined as toluene-soluble and *n*-alkane-insoluble (e.g., *n*-heptane) fraction of crude oil. Its most problematic characteristic is the poor solubility.[5] Asphaltenes comprise of hydrocarbons and components with high numbers of heteroatoms (N, O, and S), having highly condensed aromatic rings substituted with aliphatic chains. Their complexity is enhanced by the high variation in their chemical composition, molecular size, and structure, which causes one of the main problems of their analysis.[5] Characteristic features of asphaltenes are well studied,[3, 5-9] but an understanding of their structure on a molecular level has always been a challenge.[10]

In order to obtain information about such a complex mixture on a molecular level, mass spectrometry with high resolving power and high mass accuracy is essential.[11, 12] Different ionization techniques have been studied in crude oil analysis, and it has been observed that as a result of the high number of different ions present, discrimination and ion suppression effects can occur due to the high number of different ions present.[13] It is therefore not possible to obtain thorough information by simple application of one single ionization technique.[10, 12] Moreover, the structural diversity is also a key problem. Numerous isomeric compounds having different structures can be

present. As a result, there is a need to develop various separation methods for simplification prior to detection.

Prior chromatographic separation in crude oil analysis helps to reduce the complexity and eliminate discrimination effects by having less competitive species present in the mass spectrometer at the same time. Whereas the majority of the compounds are not volatile enough for gas chromatographic analysis, reversed-phase liquid chromatography accompanied by electrospray ionization is commonly used for investigation of polar compounds.[14] Normal-phase liquid chromatography is a powerful analytical tool for separation of lighter polycyclic aromatic hydrocarbons (PAH) and polycyclic aromatic sulfur heterocycles (PASH) in crude oil using, for example, a β-cyclodextrin stationary phase [15] or a polar aminocyano-bonded silica column.[16]

The most difficult crude oil fraction to study is the asphaltene fraction. Its limited solubility represents a major hurdle for the chromatographic investigation and limits the method to solvents in which asphaltenes are soluble, such as toluene, dichloromethane, chloroform, and tetrahydrofuran.[17, 18] Different separation methods have been used for analysis of asphaltene fraction, although not directly coupled to ultrahigh-resolution MS.[2, 19-21]

Argentation chromatography (also known as silver ion chromatography) has been used since the 1960s mainly for investigation of lipids.[22, 23] Nowadays, other applications, such as the separation of mixture of alkenes [24] and polycyclic aromatic hydrocarbons,[25] have become the focus of interest. The separation is based on the reversible interaction between the silver(I) ions and compounds containing π-systems via complex formation.[26-28] Silver(I) ions are immobilized by covalently bonding to the surface of a silica gel stationary phase. This prevents leaching and enables an effective substance separation.[29, 30]

In this study, we report the development of a separation method for asphaltenes using argentation chromatography. The conditions were optimized for investigation of such highly complex aromatic mixtures. By using a combination of methods, all of the benefits of the individual methods can be utilized, and the variation of the unsaturation of asphaltene components can be monitored on a molecular level.[31, 32] It is known that, depending on the ionization techniques, discrimination and suppression effects can occur. Thus, a combination of atmospheric pressure photoionization (APPI) and

atmospheric pressure chemical ionization (APCI) was chosen to widen the range of potential compounds.

5.3 Experimental section

5.3.1 Sample preparation

Asphaltenes were prepared from heavy crude from North American origin according to a modified IP 143 method using *n*-heptane (HPLC grade, Merck, Germany) for precipitation.[33] Thirty milliliters of *n*-heptane per gram of crude oil was mixed thoroughly, and the dispersed solution was refluxed for 1 h. The mixture was stored in the dark overnight and then filtered through a filter paper (Whatman, type 589.3), rinsing with hot heptane. The filter paper was placed into a Soxhlet apparatus for extraction under reflux with *n*-heptane for an additional 2 h. Then the solvent was replaced with fresh toluene, which dissolved the asphaltenes from the filter paper. In order to obtain the solid sample for investigation, toluene was removed by evaporation under vacuum.

Chemicals used for sample preparation, for synthesis, and for chromatographic separation are HPLC grade and were purchased from Sigma-Aldrich (Germany), if not stated otherwise.

For measurements, asphaltene sample (4 mg) was fully dissolved with the help of an ultrasonic bath in 1 ml of a mixture of toluene and chloroform (7:3 v/v). For direct injection, further dilution was undertaken to reach a final concentration of 250 µg/ml.

5.3.2 Argentation chromatography

The stationary phase (silver(I)-mercaptopropyl silica gel) for argentation chromatography was synthesized in our laboratory using the procedure reported by Nocun et al.[25] In order to remove adsorbed water, silica gel (10 µm LiChrosorb Si 100 (Merck, Darmstadt, Germany)) was dried at 200 °C for 24 h. A solution of 50 ml of dry toluene and 15 ml of 3-mercaptopropanotrimetoxysilane was prepared, and 10 g of silica gel was added. The toluene used in the synthesis was dried over 3 Å molecular sieves. The mixture was then stirred for 24 h at 120 °C under argon atmosphere. After filtration and washing (with dry toluene and methanol), the resulting 3-mercaptopropyl functionalized silica gel was dried at 50 °C overnight. Subsequently, the gel was stirred

in 250 ml of 0.1 M aqueous silver nitrate solution for 3 h at room temperature. After filtration and washing (with water and methanol), the resulting silver(I)-mercaptopropyl silica gel was dried again at 50 °C overnight. A stainless steel column (250 mm × 4.6 mm) was then packed with this material using a Knauer pneumatic pump at 400 bar with isopropanol (IPA). The column was then equilibrated for further measurements by washing it with cyclohexane (Acros Organics, Geel, Belgium) and dichloromethane.

Argentation chromatography was performed on an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Bremen, Germany) by injecting 50 µl of sample. A mixture of toluene and chloroform (7:3 v/v) was used as mobile phase with increasing amount of different ligands (isopropanol (IPA), acetonitrile (ACN), and dimethyl sulfoxide (DMSO)) up to 20% (v/v) at a flow rate of 2 ml/min. These solvents were used to compete with the analyte for coordination with the silver(I) ions in order to be able to separate the analyte molecules. The amount of the displacer ligands was increased stepwise during each individual separation process: after each 10 min period the concentration was set to the following percentages: 2%, 5%, 10%, 15%, and 20%, respectively. (Note: 15% and 20% of DMSO was applied for only 5 min each.)

5.3.3 Mass spectrometry and data analysis

Mass analysis was carried out on a research-type LTQ-Orbitrap Elite (Thermo Fisher Scientific, Bremen, Germany) with a resolving power of up to 480 000 at m/z 400 in absorption mode resulting in one scan roughly every 1.5 s. For ionization, a combination of atmospheric pressure photoionization (APPI) and atmospheric pressure chemical ionization (APCI) was applied. For this setup, a Syagen Krypton VUV lamp (Syagen, Tustin, CA, USA) was used with a photon emission at 10.0 and 10.6 eV and the APCI corona discharge needle was set to 4.0 µA.

Online coupling of LC/MS was achieved using PEEK capillaries directly connecting the outlet of the UV detector to the ionization source of the mass spectrometer. It was necessary to use a splitter to reduce the flow rate to 0.3 ml/min into the MS ion source. The nebulizer temperature was set to 350 °C, and nitrogen was used as sheath gas.

Positive mode MS measurements were carried out in a mass range of 200–800 using full scan mode and data were processed with the LTQ FT Ultra 2.5.5 (Thermo Fischer Scientific, Bremen, Germany).

For detailed data analysis, Xcalibur 2.2 (Thermo Fischer Scientific, Bremen, Germany) and Composer V1.0.6 software (Sierra Analytics, Modesto, CA, USA) were applied. Calculations and peak assignments were carried out under the following criteria regarding the number of possible elements: H: 0–100, C: 0– 50, N: 0–3, O: 0–3, S: 0–3, and DBE: 0–40. The calculated molecular formulas were classified into different heteroatom classes where radical cations (M) and protonated molecules (M[H]) were distinguished. For further data evaluation and graphical representation Excel (Microsoft Office Professional Plus 2010, Microsoft Excel 2010) and Origin (Origin 8.5.0) software were used.

5.4 Results and discussion

5.4.1 Method development for asphaltenes

According to earlier studies, polycyclic aromatic sulfur heterocycles can be effectively separated on a silver(I) ion-containing silica gel column due to the interaction between the silver(I) ions and the π-systems of aromatic compounds.[25]

Our aim here was to selectively analyze asphaltenes which comprise the *n*-heptane-insoluble fraction of heavy crude oil.[34] Figure 5-1 shows the mass spectrum of asphaltenes which has the most intense signals appearing between ca. 400–450. It is slightly different from the mass spectra of the *n*-heptane-soluble fraction (maltenes) and the whole crude where the highest signals appear between ca. 300–350.[3, 12, 35]

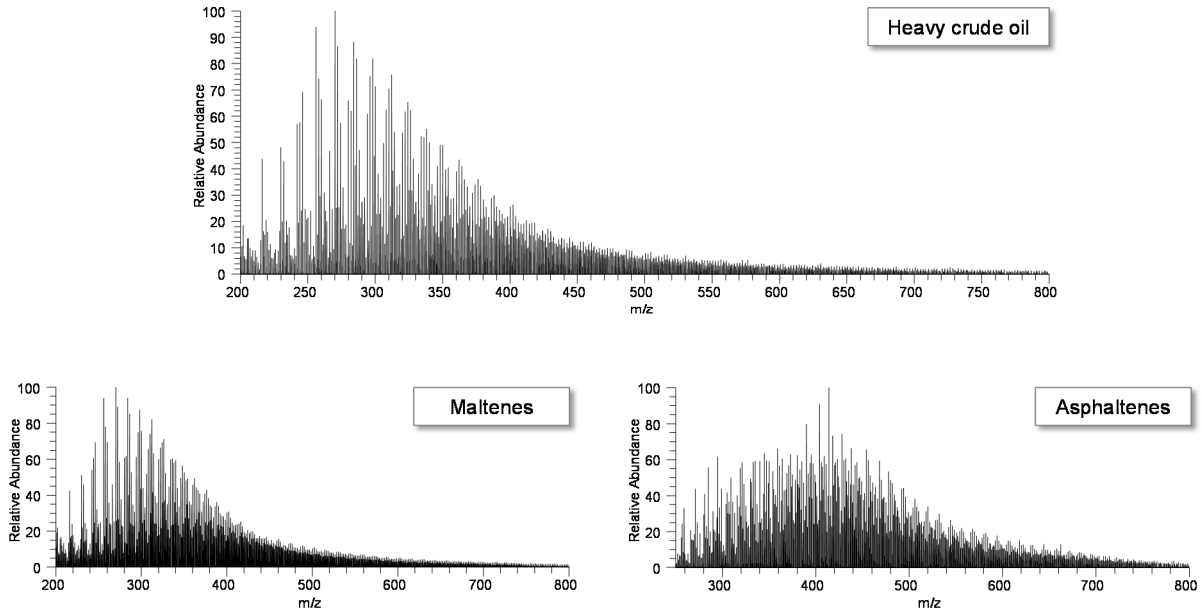

Figure 5-1. Comparison of mass spectra of *n*-heptane-soluble (maltenes) and *n*-heptane-insoluble (asphaltenes) fraction of heavy crude oil obtained from direct infusion.

The argentation chromatographic conditions as previously applied are not feasible for asphaltenes owing to their poor solubility. Our approach was to change the main solvent system. Thus, cyclohexane was replaced by toluene, and it was mixed with chloroform in the mobile phase in the chromatographic system. Because isopropanol (IPA) as competitive ligand is not strong enough to displace the interacting asphaltene compounds in the silver(I)-containing stationary phase, other competitive ligands were applied in order to reach successful elution.

Selection of the proper ligand can be critical due to the risk of irreversible interaction with the stationary phase, which we wished to avoid, as this would affect reproducibility. Figure 5-2 shows the resulting UV chromatograms at 300 nm using different competitive ligands such as IPA, acetonitrile (ACN), and dimethyl sulfoxide (DMSO), which are known solvents used for extraction of PAHs from mixtures.[36] A wavelength of 300 nm was selected for evaluation because asphaltene compounds absorb very well at this wavelength due to their condensed aromatic structure.

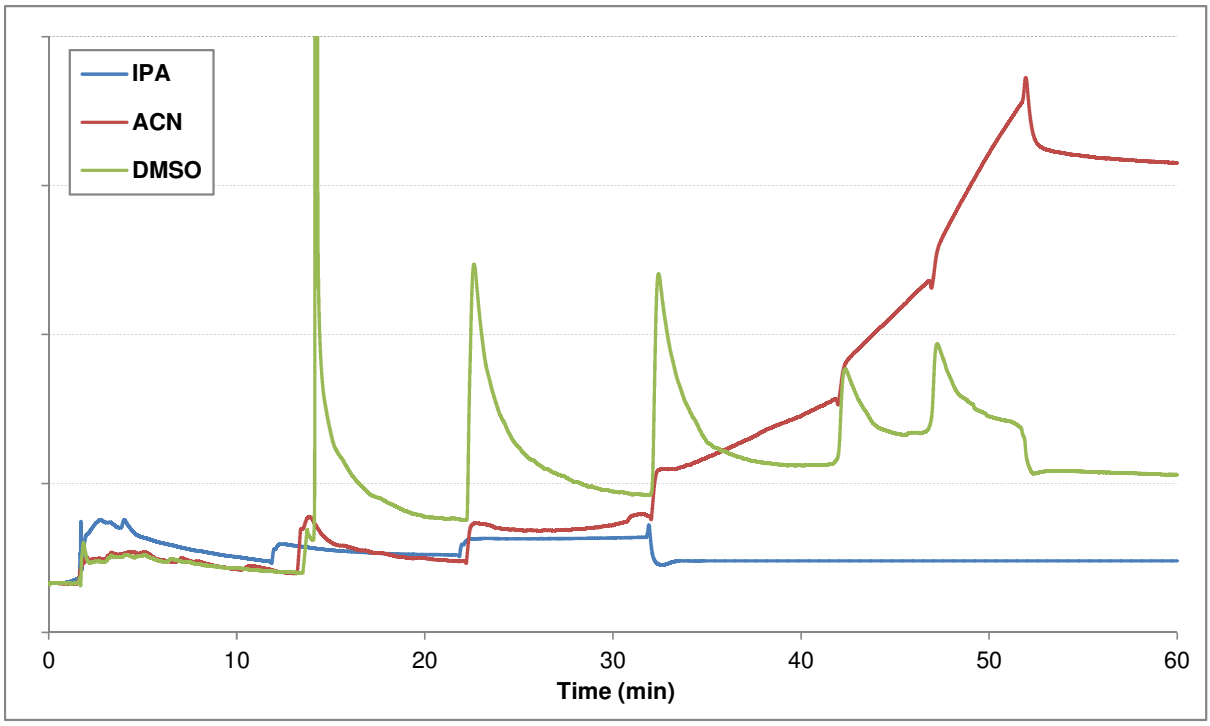

Figure 5-2. UV chromatograms at 300 nm of asphaltenes with different competitive ligands (IPA, ACN, DMSO). The concentration of these solvents was increased according to the followings: 0% in 0-10 min, 2% in 10-20 min, 5% in 20-30 min, 10% in 30-40 min, 15% in 40-45 min and 20% in 45-50 min then 0% again in 50-60 min.

As the results show here, IPA and ACN are not suitable as competitive ligands. In the case of IPA, no elution of asphaltene molecules can be observed. Using ACN results in a slightly increasing signal, which indicates a slow leaching of the compounds after 30 min (with more than 10% of ACN). However, the application of DMSO seems to be a good choice for the purpose of elution of these highly aromatic compounds. Within the first 10 min, a mixture of toluene and chloroform (7:3 v/v) was used as mobile phase, then at 10 min intervals, the amount of DMSO was increased stepwise in order to reach the following amounts: 2%, 5%, 10%, 15% and 20%. (Note: 15% and 20% of DMSO was applied only for 5 min each.) Accordingly, distinct peaks were detected at ca. 2, 15, 23, 32, 42, and 47 min. A stepwise gradient of DMSO removes compounds from the stationary phase at different intervals depending on the strength of the interactions between each compound and the stationary phase. This allows some type of sorting that is depending on these interactions and allows accumulation of compounds according to their aromaticity.

High-resolution mass spectrometry was directly coupled to the LC system to detect the compounds in each separation step at a molecular level. The compound class

distributions were calculated for each detected peak. Figure 5-3 shows the most abundant compound classes when using 0%, 2%, 5%, and 10% DMSO.

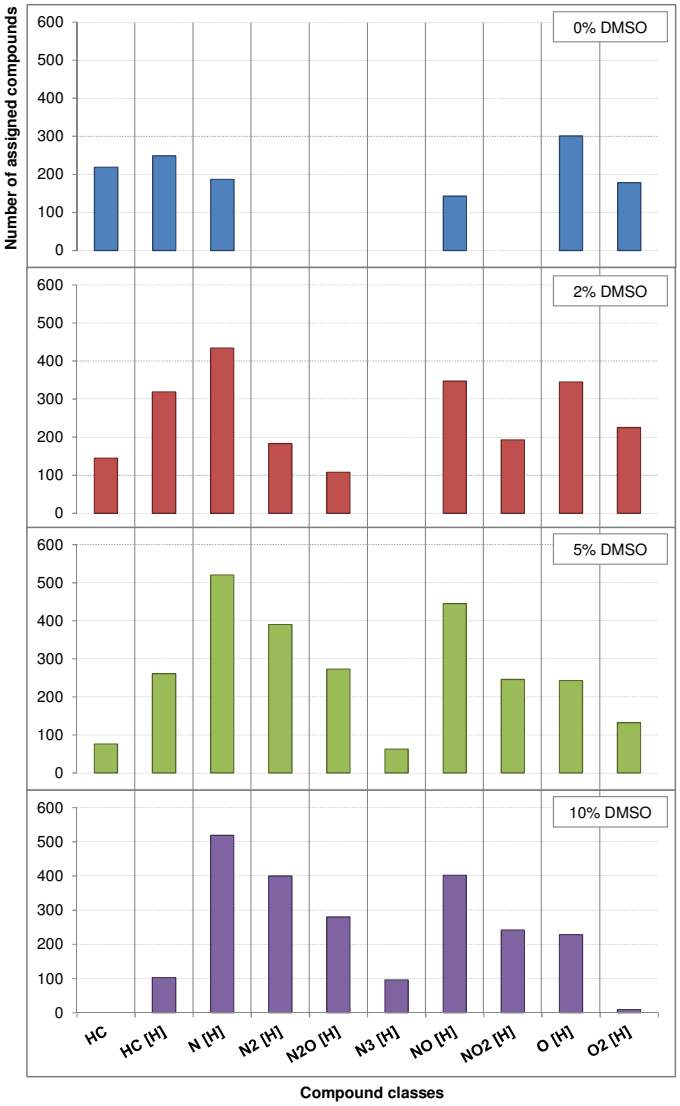

Figure 5-3. Compound class distribution based on the number of the main assigned compounds during argentation chromatographic separation of asphaltenes using toluene/chloroform (7:3 v/v) with 0%, 2%, 5% and with 10% DMSO, respectively. Note that protonated molecules are depicted with [H], while radical ions are shown without.

While the number of hydrocarbons and O_2 heteroatom class decreases and O-containing compounds do not change significantly along the separation sequence, the increase in the number of detected species containing nitrogen is clear. While only N[H] and NO[H] species can be detected using only a mixture of toluene and chloroform without DMSO, increasing DMSO to 2% results in elution of additional N_2[H], N_2O[H], and NO_2[H] species and further increase of DMSO to 5% results in elution of N_3[H]. Furthermore, when using 5% DMSO (and also up to 10%, although the increase does not show a significant change), the number of detected N[H] species is more than doubled

compared to the first fraction, and the number of N_2[H] and N_2O[H] is more than doubled compared to the second fraction.

This indicates that after injection of the sample, the majority of the free available interacting sites on the stationary phase are occupied by N-containing compounds, which can slowly elute stepwise at different stages, depending on the concentration of DMSO.

Thus, as well as the interaction between the silver(I) ions and the π-systems of the analytes, the presence of different heteroatoms and the different lengths of alkyl chains on the molecule can modify the interactions and consequently the retention time.[15] It is clear that many effects play a role during the separation. As a result of the high complexity of the system, the evaluation can therefore be fairly complicated.

Since the asphaltene sample used in these investigations contains high concentrations of nitrogen-containing compounds, as we were able to show, the N[H] heteroatom class was selected to demonstrate the effectiveness of the separation in our study. Figure 5-4 shows the respective double bond equivalents (DBE) plotted against *m/z* of all assigned N[H] species along the separation trace using toluene/chloroform (7:3 v/v) as mobile phase without DMSO, with 2% and with 5% DMSO, respectively.

DBE is a value that is representative of the degree of unsaturation of the compound and defined as the sum of the number of double bonds and ring closing bonds within a molecule. The results show that in the absence of DMSO only a minor amount of nitrogen-containing compounds with a DBE between 7 and 20 can be detected. Increasing the concentration of DMSO (2%) leads to the elution of compounds with much higher DBE values (15–35). Increasing the concentration to 5% DMSO elutes the N-compounds having even higher DBE up to 40. These data indicate that condensed aromatic structures with a DBE of up to 40 can be eluted under these conditions.[37]

Overall, prior chromatographic separation allows for a simplification of a complex sample. As a result of this approach, it is possible to detect compounds that are not observable when direct infusion into the mass spectrometer is used. One example is that in case of direct infusion, using the same conditions, only compounds with DBE less than 38 were observed while with separation DBE values of up to 40 were monitored.

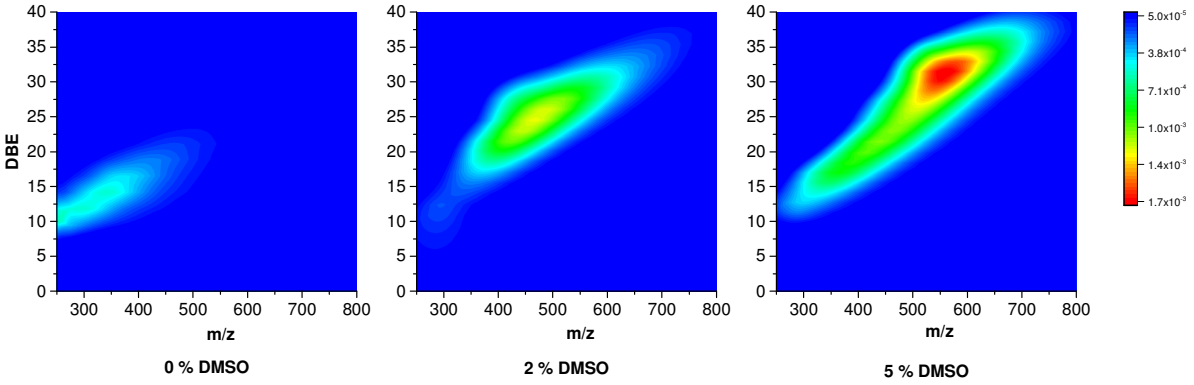

Figure 5-4. Double bond equivalents (DBE) vs. m/z distribution of nitrogen-containing compounds (N_1 class) during argentation chromatographic separation of asphaltenes using toluene/chloroform (7:3 v/v) as mobile phase without DMSO, with 2% and with 5% DMSO, respectively (relative intensity is color-coded).

5.4.2 Reconstructed ion chromatograms (RIC) and proposed structures

Mass spectrometric analysis of an analyte on a molecular level requires high resolving power and high accuracy in the determination of mass in order to be able to obtain correct peak assignments and hence determine the possible elemental compositions. In this study, argentation configuration, it is possible to follow the separation from scan-to-scan and record mass spectra in each individual scan. The analysis can be carried out not only for the full mass range but also for sufficiently small mass windows in order to follow the changes of certain selected mass traces. Figure 5-5 depicts reconstructed ion chromatograms (RIC) looking into a very narrow mass window along the separation trace. Here, three mass traces of nitrogen-containing compounds having different degrees of unsaturation (different H/C ratio) were selected. All of these N-compounds were detected in their protonated form (M[H]).

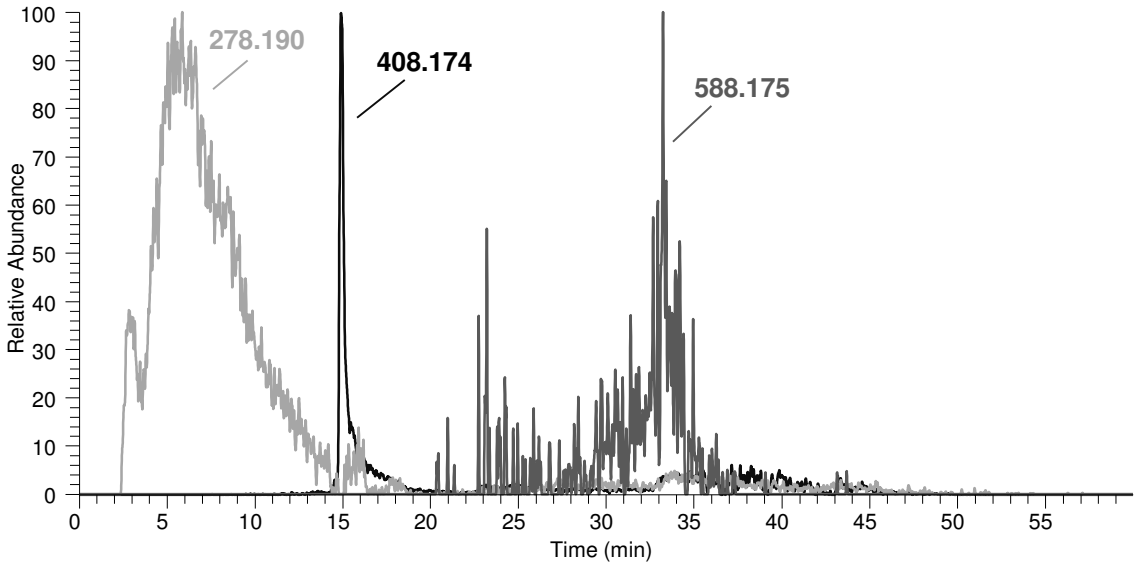

Figure 5-5. Reconstructed ion chromatograms (RIC) of nitrogen-containing protonated molecules of *m/z* of 278.190, 408.174 and 588.175 (mass tolerance window of 2 ppm) corresponding to $[C_{20}H_{24}N]^+$ with DBE 10, $[C_{31}H_{22}N]^+$ with a DBE 22 and $[C_{46}H_{22}N]^+$ with a DBE 37, respectively. The intensities for the signals are 1.7*104, 1.3*105 and 4.5*103.

In this example, the mass trace from the ion at *m/z* 278.190 corresponds to $[C_{20}H_{24}N]^+$, which has the highest H/C ratio (most saturated), and it is eluted at the beginning of the separation without using any competitive ligand. This compound is relatively small. The fairly broad peak can possibly be explained by the relatively small size of the molecule, which can facilitate secondary interactions with the surface material (e.g., dipole–dipole interactions with the unreacted silanol groups) causing the tailing. The mass trace from the ion at *m/z* 408.174 corresponds to $[C_{31}H_{22}N]^+$ which has higher unsaturation, resulting in it being retained longer on the surface of the column. With 15 min retention time and 2% of DMSO, most of the compound is eluted simultaneously, and this results in a very sharp peak. Finally, the mass trace from the ion at *m/z* 588.175 $[C_{46}H_{22}N]^+$, which has the highest unsaturation of the three compounds and hence is the most condensed structure. It is eluted in the later section of the separation trace using 5–10% DMSO with a slightly broader time range. These results indicate that asphaltene compounds having different degrees of unsaturation can be separated depending on the amount of competitive ligand used during the separation.

Compounds, especially those with fairly high unsaturation with the same chemical composition, can be present with different constitutional structures. These constitutional structures can have very different chemical and physical properties and are detected at different stages of the separation, as it is shown in Figure 5-6.

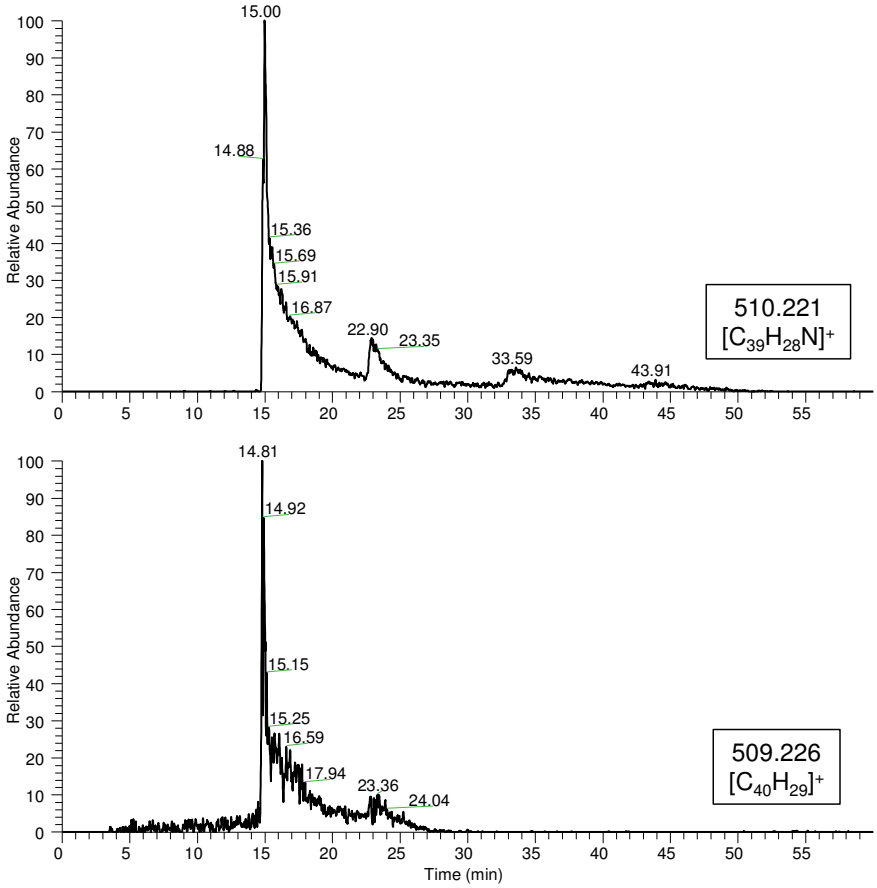

Figure 5-6. RIC of *m/z* of 510.221 and 509.226 (mass tolerance window of 2 ppm) corresponding to the formulas $[C_{39}H_{28}N]^+$ and $[C_{40}H_{29}]^+$.

The multiple signals along the separation trace refer to the compounds with the same chemical composition but different structures and are therefore present as isomers. Clearly, isomers with various types of structures will have different active sites that will interact differently with the silver(I) ions on the surface of the stationary phase. For example, the mass trace from the ion at *m/z* 510.221 corresponds to the compound with the elemental formula of $C_{39}H_{27}N$ (detected in its protonated form as $[C_{39}H_{28}N]^+$), which can exist in different types of isomeric structures (some of them are shown in Figure 5-7). This formulation can manifest itself in different functionalities, such as carbazole derivatives, indole derivatives, structures possessing only 6-membered rings, or highly condensed aromatic structures with fused cores. Even if the isomers have the same degree of unsaturation, their 3D structure can be very diverse, exhibiting, for example, basic or non-basic features. Thus, basicity and steric effects will affect the nature of complex formation with the substrate, its strength of interaction and hence its stability.

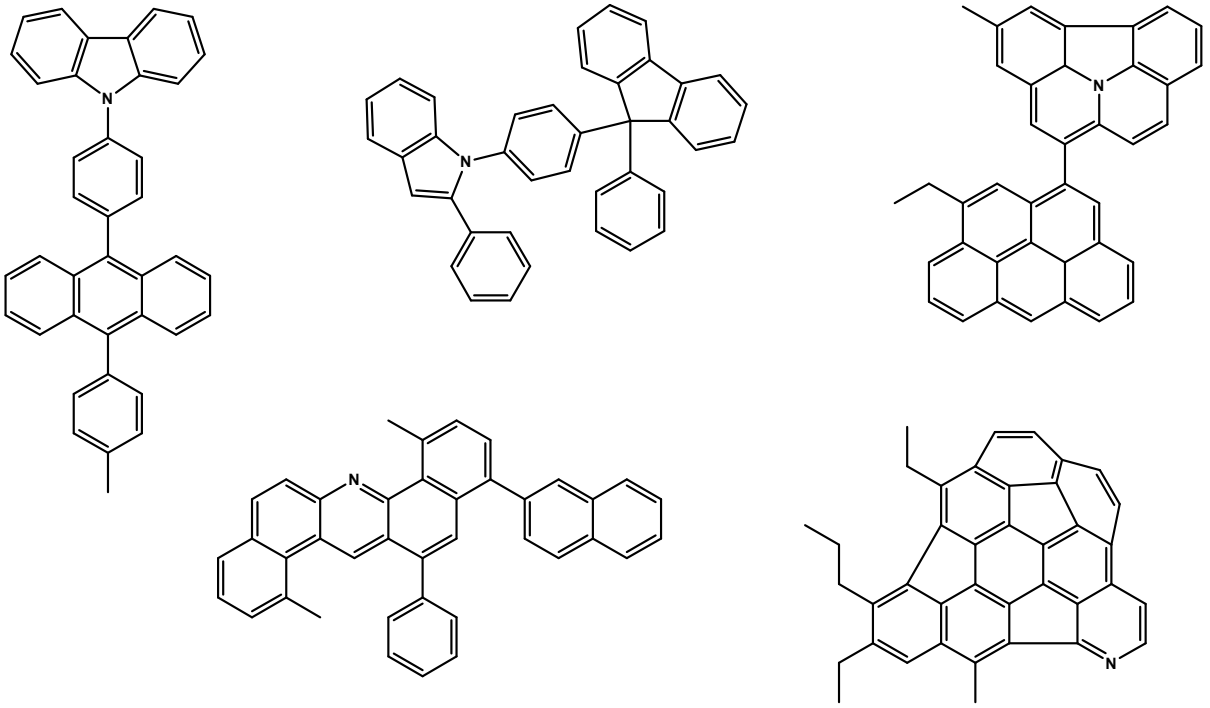

Figure 5-7. Some possible structures for chemical compounds with a formula $C_{39}H_{27}N$.

These results demonstrate that the combination of argentation chromatographic separation and mass spectrometry with high resolution makes the detailed investigation of highly aromatic asphaltene compounds possible and can provide additional structural information. Unfortunately, the isomeric compounds in asphaltene with their high aromaticity are not easily available as commercial standards, so in order to further understand their behavior, it will be necessary to synthesize them.

5.5 Conclusion

A new selective chromatographic method has been developed for the analysis of crude oil asphaltenes. It uses argentation chromatography, whereby the separation is based on the complex formation ability of silver(I) ions and π-systems of unsaturated compounds depending on the composition of the mobile phase. Elution with toluene/chloroform (7:3 v/v) mixtures has proven to be effective, especially in combination with DMSO as competitive ligand. The concentration of DMSO is critical. This method overcomes the main difficulties of asphaltene analysis due to its high complexity and poor solubility, making the method an effective tool for asphaltene investigation.

Combined with online coupling to high-resolution mass spectrometry, the effectiveness of the separation can be monitored on a molecular level. Isomeric compounds can be separated using this approach. Their different structural features (e.g., basic or non-basic, fused or non-fused core) have different strengths of interaction with the silver(I) ions due to steric effects, accessibility to the π-systems and the nature of the heteroatoms. Highly unsaturated, polycondensed heterocycles possessing DBE up to 40 have been detected.

5.6 References for Chapter 5

[1] E. C. Sanford. Interrelationship Between Oil Sand Fine Solids, Process Aids, Mechanical Energy and Oil Sand Age after Mining. *Can. J. Chem. Eng.* **1983**, *61*, 554-567.

[2] M. M. Boduszynski. Composition of Heavy Petroleums. 2. Molecular Characterization. *Energy Fuels* **1988**, *2*, 597–613.

[3] O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall. Asphaltenes, Heavy Oils, and Petroleomics, Springer, New York, **2007**.

[4] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Characterization of Saturates, Aromatics, Resins, and Asphaltenes Heavy Crude Oil Fractions by Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2012**, *26*, 3481-3487.

[5] O. C. Mullins. The Modified Yen Model. *Energy Fuels* **2010**, *24*, 2179-2207.

[6] P. Arpino. Combined liquid chromatography mass spectrometry. Part I. Coupling by means of a moving belt interface. *Mass Spectrom. Rev.* **1989**, *8*, 35-55.

[7] E. Y. Sheu. Petroleum Asphaltenes - Properties, Characterization, and Issues. *Energy Fuels* **2002**, *16*, 74-82.

[8] H. Groenzin, O. C. Mullins. Molecular Size and Structure of Asphaltenes from Various Sources. *Energy Fuels* **2000**, *14*, 677-684.

[9] L. Robert B, in *Chemistry of Asphaltenes, Vol. 195*, American Chemical Society, **1982**, pp. 17-27.

[10] S. K. Panda, J. T. Andersson, W. Schrader. Characterization of Supercomplex Crude Oil Mixtures: What Is Really in There? *Angew. Chem. Int. Ed.* **2009**, *48*, 1788-1791.

[11] R. Rodgers, A. Marshall, in *Asphaltenes, Heavy Oils, and Petroleomics* (Eds.: O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall), Springer New York, **2007**, pp. 63-93.

[12] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Impact of Different Ionization Methods on the Molecular Assignments of Asphaltenes by FT-ICR Mass Spectrometry. *Anal. Chem.* **2012**, *84*, 5257-5267.

[13] P. Schmitt-Kopplin, M. Englmann, R. Rossello-Mora, R. Schiewek, K. J. Brockmann, T. Benter, O. J. Schmitz. Combining chip-ESI with APLI (cESILI) as a multimode source for analysis of complex mixtures with ultrahigh-resolution mass spectrometry. *Anal. Bioanal. Chem.* **2008**, *391*, 2803-2809.

[14] Y. Zhang, C. Xu, Q. Shi, S. Zhao, K. H. Chung, D. Hou. Tracking Neutral Nitrogen Compounds in Subfractions of Crude Oil Obtained by Liquid Chromatography Separation Using Negative-Ion Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2010**, *24*, 6321-6326.

[15] S. K. Panda, W. Schrader, J. T. Andersson. β-Cyclodextrin as a stationary phase for the group separation of polycyclic aromatic compounds in normal-phase liquid chromatography. *J. Chromatogr. A* **2006**, *1122*, 88-96.

[16] S. Lababidi, S. K. Panda, J. T. Andersson, W. Schrader. Direct Coupling of Normal-Phase High-Performance Liquid Chromatography to Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for the Characterization of Crude Oil. *Anal. Chem.* **2013**, *85*, 9478-9485.

[17] S. Sato, T. Takanohashi. Molecular Weight Calibration of Asphaltenes Using Gel Permeation Chromatography/Mass Spectrometry. *Energy Fuels* **2005**, *19*, 1991-1994.

[18] S. Dong, A. M. Striegel. Size-Exclusion Chromatography of Asphaltenes: An Experimental Comparison of Commonly Used Approaches. *Chromatographia* **2013**, *76*, 725-733.
[19] A. W. Lynch, M. G. Thomas. Asphaltene analysis using size exclusion chromatography. *Fuel Process. Technol.* **1983**, *8*, 13-18.
[20] T. N. Loegel, N. D. Danielson, D. J. Borton, M. R. Hurt, H. I. Kenttämaa. Separation of Asphaltenes by Reversed-Phase Liquid Chromatography with Fraction Characterization. *Energy Fuels* **2012**, *26*, 2850-2857.
[21] E. Al-Muhareb, T. J. Morgan, A. A. Herod, R. Kandiyoti. Characterization of Petroleum Asphaltenes by Size Exclusion Chromatography, UV-fluorescence and Mass Spectrometry. *Pet. Sci. Technol.* **2007**, *25*, 81-91.
[22] L. J. Morris. Separations of lipids by silver ion chromatography. *J. Lipid Res.* **1966**, *7*, 717-732.
[23] R. O. Adlof. Normal-phase separation effects with lipids on a silver ion high-performance liquid chromatography column. *J. Chromatogr. A* **1997**, *764*, 337-340.
[24] J. C. Aponte, J. T. Dillon, R. Tarozo, Y. Huang. Separation of unsaturated organic compounds using silver–thiolate chromatographic material. *J. Chromatogr. A* **2012**, *1240*, 83-89.
[25] M. Nocun, J. T. Andersson. Argentation chromatography for the separation of polycyclic aromatic compounds according to ring number. *J. Chromatogr. A* **2012**, *1219*, 47-53.
[26] S. Winstein, H. J. Lucas. The Coordination of Silver Ion with Unsaturated Compounds. *J. Am. Chem. Soc.* **1938**, *60*, 836-847.
[27] J. S. Dewar. A review of the pi-complex theory. *Bull. Soc. Chim. Fr.* **1951**, *18*, C71-C79.
[28] J. G. Traynham, J. R. Olechowski. Complexation of Cyclic Olefins with Aqueous Silver Ion and with Molecular Iodine1,2. *J. Am. Chem. Soc.* **1959**, *81*, 571-574.
[29] J. C. Aponte, J. T. Dillon, Y. Huang. The unique liquid chromatographic properties of Group 11 transition metals for the separation of unsaturated organic compounds. *J. Sep. Sci.* **2013**, *36*, 2563-2570.
[30] J. A. Dunn, K. B. Holland, J. R. Jezorek. Argentation liquid chromatography of polynuclear aromatic hydrocarbons on a silver(l)-loaded mercaptopropyl silica gel stationary phase. *J. Chromatogr. A* **1987**, *394*, 375-381.
[31] A. Makarov, M. Scigelova. Coupling liquid chromatography to Orbitrap mass spectrometry. *J. Chromatogr. A* **2010**, *1217*, 3938-3945.
[32] S. Lababidi, W. Schrader. Online normal-phase high-performance liquid chromatography/Fourier transform ion cyclotron resonance mass spectrometry: Effects of different ionization methods on the characterization of highly complex crude oil mixtures. *Rapid Commun. Mass Spectrom.* **2014**, *28*, 1345-1352.
[33] H. Alboudwarej, J. Beck, W. Y. Svrcek, H. W. Yarranton, K. Akbarzadeh. Sensitivity of Asphaltene Properties to Separation Techniques. *Energy Fuels* **2002**, *16*, 462-469.
[34] T. Fan, J. S. Buckley. Rapid and Accurate SARA Analysis of Medium Gravity Crude Oils. *Energy Fuels* **2002**, *16*, 1571-1575.
[35] W. C. Hockaday, A. M. Grannas, S. Kim, P. G. Hatcher. The transformation and mobility of charcoal in a fire-impacted watershed. *Geochim. Cosmochim. Acta* **2007**, *71*, 3432-3445.

[36] C. F. Poole, T. Karunasekara, T. C. Ariyasena. Totally organic biphasic solvent systems for extraction and descriptor determinations*. J. Sep. Sci.* **2013**, *36*, 96-109.

[37] A. M. McKenna, A. G. Marshall, R. P. Rodgers. Heavy Petroleum Composition. 4. Asphaltene Compositional Space*. Energy Fuels* **2013**, *27*, 1257-1267.

CHAPTER 6.

COMBINATION OF ANALYTICAL TECHNIQUES FOR STRUCTURAL ELUCIDATION OF INDIVIDUAL CHEMICAL COMPOUNDS IN EXTREMELY COMPLEX SAMPLES

Redrafted from "L. M. Guricza, W. Schrader. Combination of analytical techniques for structural elucidation of individual chemical compounds in extremely complex samples", will be submitted to Analytical Chemistry.

6.1 Abstract

Study on complex mixtures requires the combination of multiple analytical techniques to achieve information with sufficient details. Mass spectrometry with ultrahigh resolution provides information on a molecular level, however, without any separation method some compounds might not be detected due to ion suppression and discrimination effects. In order to get a full description of complex samples implication of several separation steps is necessary. Crude oil is one of the most complex mixtures known with estimates of more than one million different compounds present. The ability of gaining detailed structural information about such a complex mixture is limited by the complexity. The combination of size exclusion chromatography followed by argentation chromatography helps reducing the complexity by accomplishing the separations based on certain properties of the compounds (size and number of π-bonds and heteroatoms) and eliminates matrix effects. The application of these simplification and separation procedures results in a much simpler sample that is feasible to provide structural elucidation via fragmentation measurements which was investigated in complex crude oil asphaltenes in this study. The fragmentation pattern demonstrates that the dominant structure is island type judging from the loss of various alkyl chains.

6.2 Introduction

The phrase complex in the field of analytical chemistry gives a first impression that the analysis is a challenging task, especially if we are dealing with an analyte which consists of more than one million different compounds.[1] Studying chemical transformations from reactions in such complex samples is not an easy task, especially when extreme molecular changes occur.[2] Crude oil is one of the most complex samples occurring in the nature.[3] An in-depth knowledge is necessary about the containing compounds regarding not only their bulk properties such as boiling-point distribution and viscosity but also their characteristics on a molecular level, most importantly their structure. This knowledge is very important for the oil industry where problems such as precipitation frequently occur and cause significant damage during transportation and refining processes.[4] The goal is, however, not only to circumvent any potential damage but also to improve and optimize the current technology. Development of chemical processes such as hydrotreating and hydrocracking are essential to convert heavier fractions of crude oil into more valuable products e.g. light hydrocarbons.[5, 6]

Crude oils consist of compounds with a broad molecular weight distribution (usually ranging from around 50 to ca. 1200 Da) depending on the boiling point, polarity (from non-polar to polar with mostly N, O and/or S heteroatoms) and acidity (acidic, neutral or basic) which all together demonstrate its high complexity.[7-10] For a full characterization including structural information, various analytical methods are required describing the samples from different aspects. Methods such as IR, NMR or fluorescence measurements can provide information about the bulk sample; however, the description of the individual molecules is considered to be even more important. When the question of interest is to get information about the molecular structure, the possible tools are limited. Solid state ^{13}C NMR and X-ray crystallography are the relevant methods, however, provide information only about the bulk properties. These are mostly used to estimate the average amount of aromatic and aliphatic carbon atoms and average fused aromatic ring size in the asphaltene mixture.[11] The only alternative for structural elucidation is mass spectrometry, namely MS/MS and MS^n measurements that are capable of providing structural information on a molecular level. One major difficulty only is that for MS^n measurements a certain mass window has to be isolated. This is usually not a big problem as has been done on routine basis in pharmaceutical

research, including proteomics, metabolomics and bioanalysis.[12, 13] But it can become a real problem when the isolation window does not contain one or two different ions but up to 200. Then the interpretation of the fragmentation is hardly possible.

On the other side, mass spectrometry alone detects the distinct signals of the charged species representing different molecular compositions[14] with the exact chemical formulae that can be calculated from Fourier transform based measurement.[15] However, using MS in full scan mode provides no further chemical information besides the elemental compositions; thus isomeric compounds cannot be differentiated. Detailed structural information cannot be gained by using mass spectrometry alone.

These above mentioned problems can be reduced and minimized if a smaller number of different species is present at a certain time in the analyzer cell. Therefore, simplification of the complex sample is an inevitable step. Several chromatographic methods based on targeted characteristics have been used for heavy crude oil analysis. Ligand exchange chromatography with palladium(II) ion-based stationary phase has been proven to be an effective technique for separation of heteroatom-containing molecules such as polycyclic aromatic sulfur compounds.[16] Other approaches use β-cyclodextrin [17] or silver(I) ion-bonded silica gel [18, 19] as stationary phase which can separate aromatic compounds based on the number of aromatic rings. The separation according to molecular weight in case of crude oil asphaltenes was investigated with size exclusion chromatography (SEC) and the different approaches regarding the solvent system were compared.[20] Normal-phase liquid chromatography using a polar aminocyano stationary phase coupled to mass spectrometry has been presented to separate isomeric crude oil compounds, basic and non-basic N-heterocycles.[21]

The combination of several separation methods - starting from SARA fractionation [22, 23] - leads to a considerably simpler sample. In this study the simplification experiments were demonstrated by the *n*-heptane-insoluble fraction of crude oil, namely asphaltenes. This is the heaviest and most aromatic fraction and consists of polycondensed aromatic structures with relatively high number of heteroatoms.[24, 25] Chromatographic separation of asphaltenes is extremely challenging because besides of the complexity, their solubility is also a huge concern.[26] SEC and argentation chromatography have been reported to be the methods of choice for chromatographic separation prior to MS for asphaltenes. Therefore, the combination of these two methods was applied here. Application of two

consecutive chromatographic techniques is a new approach for investigation of crude oil asphaltenes.

While in case of the whole sample a non-targeted analysis has to be accomplished by looking for homologous series instead of individual molecules, the high level of simplification allows us to select targets for further investigation such as MS/MS measurements. In this study it is demonstrated that the combination of chromatographic methods followed by MS/MS measurements is a benefit for structural elucidation of complex mixtures such as crude oil asphaltenes.

6.3 Experimental section

6.3.1 Sample preparation

Asphaltenes were prepared from a heavy crude oil from a North American source according to a modified IP 143 method.[27] For direct infusion, the solid asphaltene sample was sonicated for 20 minutes in a mixture of toluene and chloroform (7:3 v/v), diluted to a final concentration of 250 µg/ml and then analyzed without any further treatment.

For SEC measurement, asphaltene sample was diluted in tetrahydrofuran (THF) to a final concentration of 2 mg/ml and sonicated for 20 minutes. Fraction collection was accomplished in 30 seconds segments from 19 to 27 minutes. The solvent of the fractions was evaporated to dry on a rotary evaporator. For further argentation chromatographic measurements, selected fractions were dissolved in a mixture of toluene and chloroform (7:3 v/v) resulting in a concentration of ca. 500 µg/ml. These samples were injected into the argentation chromatographic system and fraction collection was carried out based on the stepwise increasing dimethyl sulfoxide concentration (0, 2, 5, 10, 15 and 20%) in every 10 minutes. The resulting samples were concentrated to ca. 0.5 ml and then analyzed by mass spectrometry.

6.3.2 Instruments and methods

Size exclusion chromatography was performed on an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) by injecting 100 µl sample. A combination of styrene-divinyl benzene copolymer analytical columns (300 x 8.0 mm ID, 5 µm particle size) (PSS, Mainz, Germany) with pore sizes of 10^3 Å and 10^2 Å was used as

stationary phase and THF as mobile phase at a flow rate of 1 ml/min. The stationary phase for argentation chromatography was silver(I)-mercaptopropyl silica gel synthesized in our laboratory based on previous studies.[18] The measurement was performed using an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Bremen, Germany) by injecting 100 µl sample. A mixture of toluene and chloroform (7:3 v/v) was used as mobile phase with stepwise increasing amount of dimethyl sulfoxide (DMSO) according to the following: 0% (0-10 min), 2% (10-20 min), 5% (20-30 min), 10% (30-40 min), 15% (40-45 min), 20% (45-50 min) and returned to 0% (50-60 min). The flow rate was 2 ml/min.

Mass spectra were recorded on a research-type Orbitrap Elite mass spectrometer (Thermo Fischer Scientific, Bremen, Germany). The mass spectra were recorded in a range of 200-900 using full scan mode at a mass resolving power of 480 000 (FWHM at *m/z* 400). Ionization was achieved using an atmospheric pressure photoionization (APPI) source in positive mode with a Krypton VUV lamp (Syagen Technologies, Tustin, CA, USA) with a photon emission at 10.0 and 10.6 eV. The heated sprayer was operated at 350 °C, nitrogen was used as sheath gas and the flow rate was 10 µl/min.

Collision-induced dissociation (CID) was used for fragmentation measurements and the parent mass was selected with an isolation window of 1.0 Da. The isolated ions were recorded and then kinetically excited with medium (30%) and high (45-50%) energy (in case of Thermo Scientific LTQ instruments it is called normalized collision energy) using helium as collision gas.

6.3.3 Data analysis

The acquired data were analyzed by Xcalibur 2.2 software (Thermo Fisher Scientific, Bremen, Germany). For peak assignments the following criteria regarding the number of possible elements and the number of double bond equivalent (DBE) were applied: $0 < H < 1000$, $0 < C < 200$, $0 < N < 3$, $0 < O < 3$, $0 < S < 3$, and $0 < DBE < 40$ with a maximum mass tolerance of 1 ppm.

During evaluation, DBE is used as an important parameter for comparison of the unsaturation degree of the components. DBE refers to the sum of the rings and the number of double bonds within a molecule and is calculated from the molecular formula

($C_cH_hN_nO_oS_s$) of the assigned individual peaks by the following equation: $DBE = c - \frac{h}{2} + \frac{n}{2} + 1$.

6.4 Results and discussion

The full mass spectrum of crude oil asphaltenes shows a distribution in the mass range of 200-900 in positive mode with APPI ionization which can be seen in Figure 6-1 (top). The complexity can be demonstrated by mass scale-expanded views to randomly selected mass windows, e.g. at *m/z* 337 and 422 (Figure 6-1, center and bottom). The most important peak assignments and calculated DBE values are shown here.

Within 0.12 Da mass units up to 20 peaks can be assigned. These are for instance hydrocarbons in their protonated form ($C_{26}H_{25}$), hydrocarbons as radical cations ($C_{33}H_{26}$) or as an ^{13}C-isotope ($C_{25}{}^{13}CH_{24}$), heteroatom-containing compounds in their protonated form ($C_{24}H_{17}S$, $C_{25}H_{21}O$, $C_{32}H_{24}N$) or as heterocyclic radical cations ($C_{31}H_{18}S$, $C_{24}H_{19}ON$, $C_{32}H_{22}O$, $C_{25}H_{23}N$), while some isotopic signals of compounds with heteroatoms are present as well. This representation confirms the necessity of simplification, especially if the goal is to gain structural information.

Application of chromatographic methods enables the separation of the complex analyte based on selected properties. The effectiveness of the separations is already remarkable by looking at the full mass spectra even without any details. In Figure 6-2 the different segments of the total ion chromatograms (TIC) show various mass distributions. In size exclusion chromatography it is kind of expected because the separation is based on the molecular size of the compounds. Here at the earlier stage of the separation (21-21.5 min) the most intensive peaks of the distribution appear at *m/z* 550, while in the time range of 24-24.5 min the detected mass range is shifted towards the lower values with the most intensive peaks around *m/z* of 350 (Figure 6-2A). Although, argentation chromatography separates the molecules based on their unsaturation and aromaticity independently from the mass range, the mass spectra of selected segments also show differences. Comparing the mass spectra in the time range of 0-10 min the most intensive peaks are around *m/z* 300, while in the time range of 10-20 min the highest peaks appear at *m/z* 450 (Figure 6-2B).

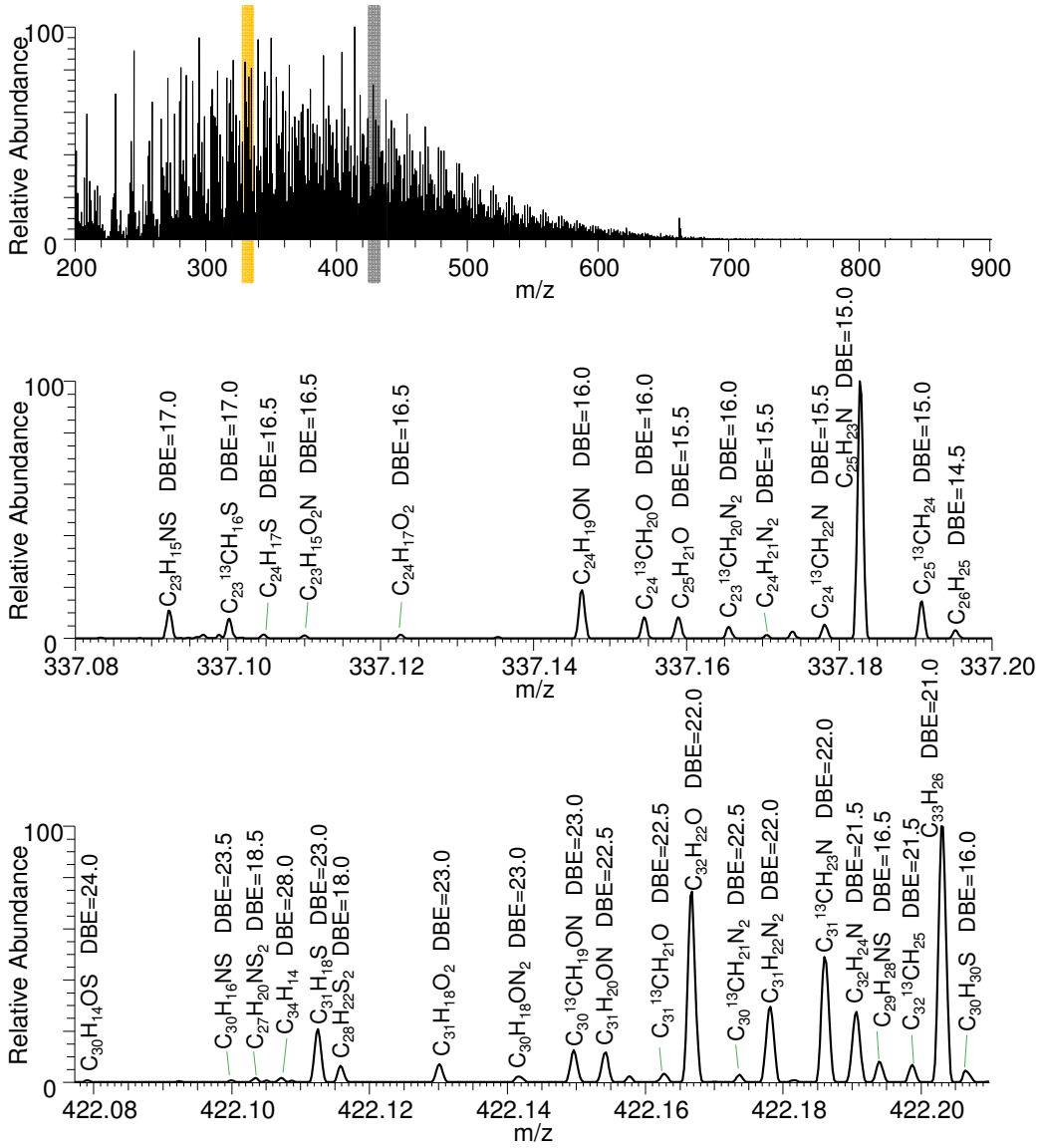

Figure 6-1. Mass spectrum of crude oil asphaltenes in full scan mode (top) and mass scale-expanded views in the mass range of 337.08-337.20 (center) and 422.08-422.20 (bottom) with the most important assignments and the calculated DBE values for each. Note: when a calculated DBE of the given molecular formula is an integer that means that the compound is detected in radical cation form, when the DBE value is not an integer (it ends to .5) then the compound is protonated.

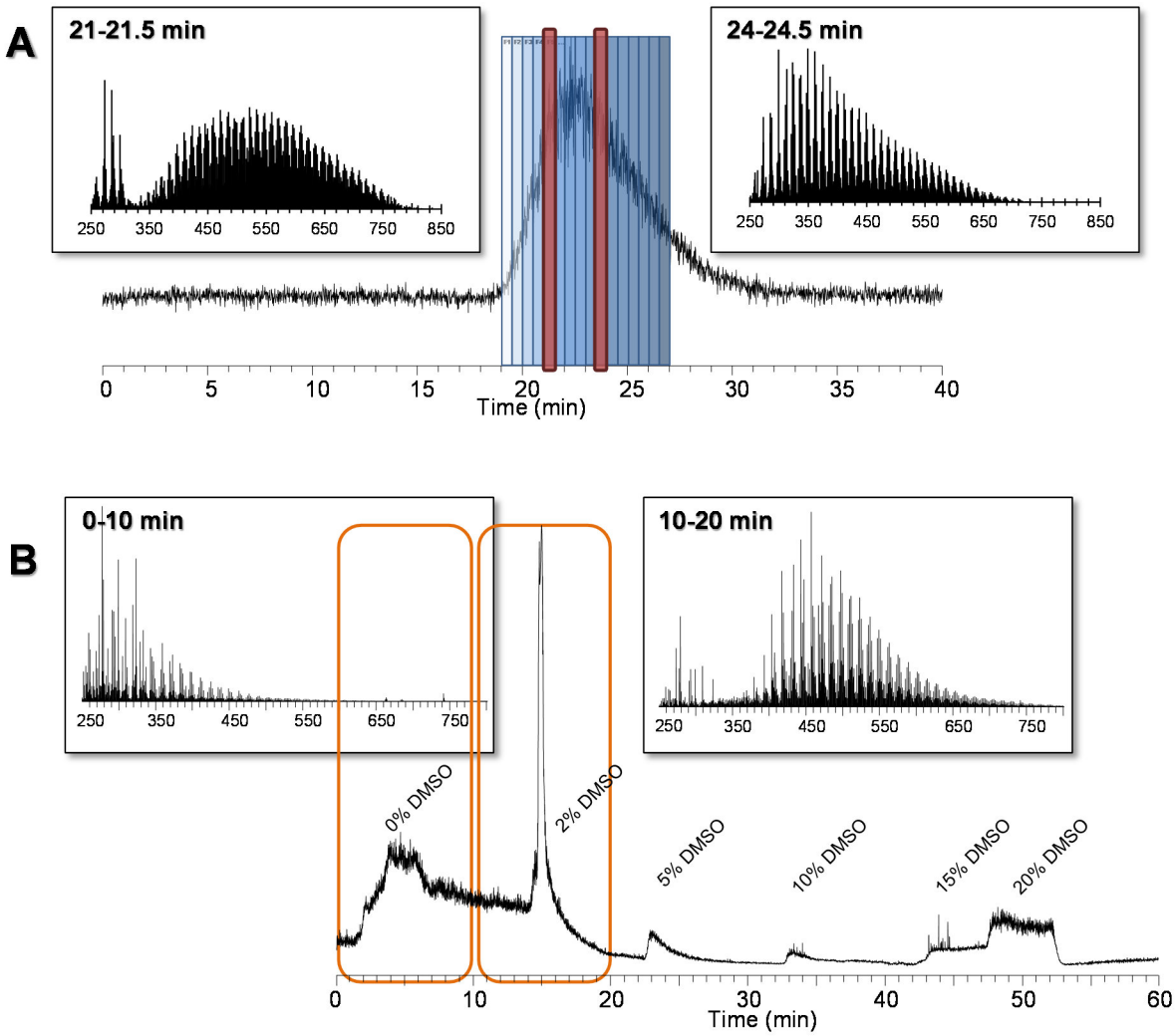

Figure 6-2. Total ion chromatogram (TIC) of asphaltenes size exclusion chromatographic (SEC) separation together with mass spectra in two selected time ranges (A) and TIC of argentation chromatographic separation together with mass spectra at two selected time segments (B).

This leads to the idea of using the combination of these two methods. It is clear that the more chromatographic separation is applied, the simpler the sample becomes. However, a meaningful approach needs to be found for sample preparation because it is fairly time consuming. Each separation step involves multiple times of fraction collections plus evaporation of the solvents and includes a high degree of dilution of the analyte. In this study two consecutive separations are performed on asphaltenes, first SEC then argentation chromatography. Figure 6-3 shows the effect of the separation comparing the mass scale-expanded views of the original sample (top), one fraction after SEC separation (center) and one fraction after two chromatographic separations (SEC and argentation chromatography) (bottom) at *m/z* 364 nominal mass. The changes of the detected peaks are apparent. The original sample contains a hydrocarbon $C_{29}H_{16}$ (DBE=22) as the most intensive peak and other compounds (S-, OS-, S_2-, O-, O_2-, ON-, N-

and N_2-species) are also present but suppressed and therefore present in lower intensities. After the application of SEC, the selected fraction (between 24 and 24.5 min) does not contain this $C_{29}H_{16}$ hydrocarbon compound therefore the others are not suppressed and can appear with increased intensities and also new ones can be additionally detected. This demonstrates that although a simplification step was applied here, the number of detected peaks is not necessarily less, but due to the elimination of ion suppression a more detailed information depth can be achieved. Furthermore, as the separation is based on size, isomers might be separated here because the same composition, especially with high number of carbon atoms, might represent different structures: either a compound with extended size or a compact molecule. After application of the second separation step (argentation chromatography), the selected fraction (between 20 and 30 min) shows apparently less peaks, mostly N-, NO-, NO_2- and NS-compounds in the same mass range. So the simplification provided by the two consecutive separation steps has been successfully accomplished. The resulting simplified fractions contain fewer amounts of compounds which have similar characteristics regarding molecular size, aromaticity and heteroatom content.

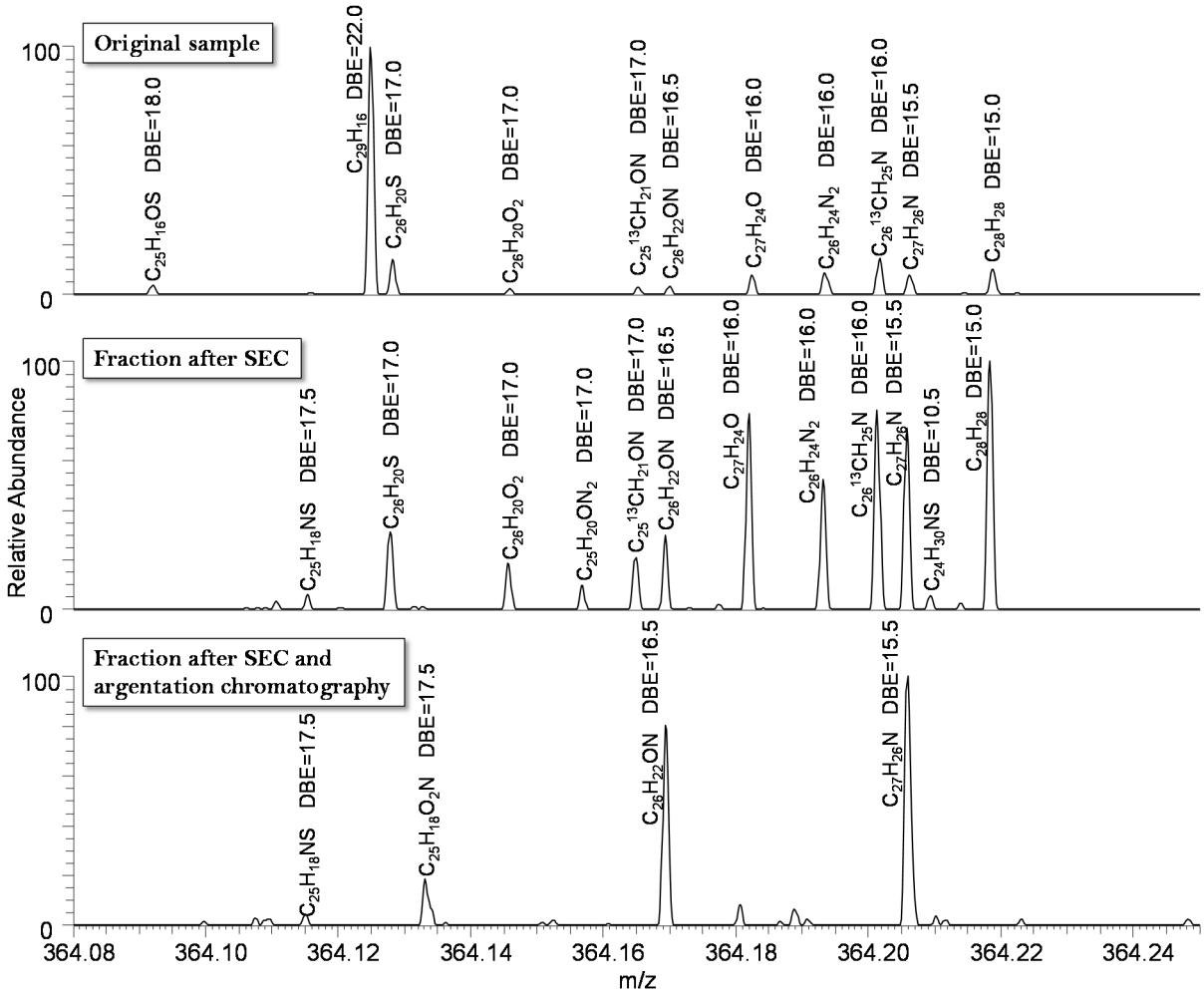

Figure 6-3. Comparison of mass scale-expanded views of original asphaltene sample (top), one fraction after SEC separation (center) and one fraction after SEC and argentation chromatography (bottom).

The result of the simplification procedure makes it possible to perform structural determination experiments with deeper insight. Owing to the high-resolution mass spectrometric measurements the fragment peaks can be assigned very accurately. The fragmentation experiment with crude oil asphaltenes at *m/z* 337 shows a fragmentation pattern of a nitrogen-containing compound with decaying intensities having a difference of 15 and 14 *m/z*, consecutively (Figure 6-4). This corresponds to the loss of alkyl chains (methyl, ethyl, propyl, etc.) of the selected compounds. Previous studies show that asphaltene model molecules fragment predominantly by losing their alkyl chains – typically all but one carbon atom of the chain.[28, 29] In general such a spectrum provides us information about the maximum total number of alkyl side chains from the number of peak serial corresponding to one heteroatom class. Furthermore the last detected peak within one serial can foreshadow the possible smallest core size.[28, 29] In case of asphaltenes fragmentation of molecular ions of *m/z* 337 these loss of alkyl

chains can be clearly observed, however there are only a few number of fragment peak series referring to the fact that the possible alkyl chains present in the molecules are not too long (maximum 4-5 carbon long). There are no fragment peaks below *m/z* 280 therefore the possible smallest core is presumably fairly big containing 5-7 condensed aromatic rings. Similar fragmentation pattern can be observed in case of other selected molecular ions. These results confirm that the dominant structures of asphaltenes are island type – one condensed aromatic core substituted with alkyl chains.[28, 30, 31]

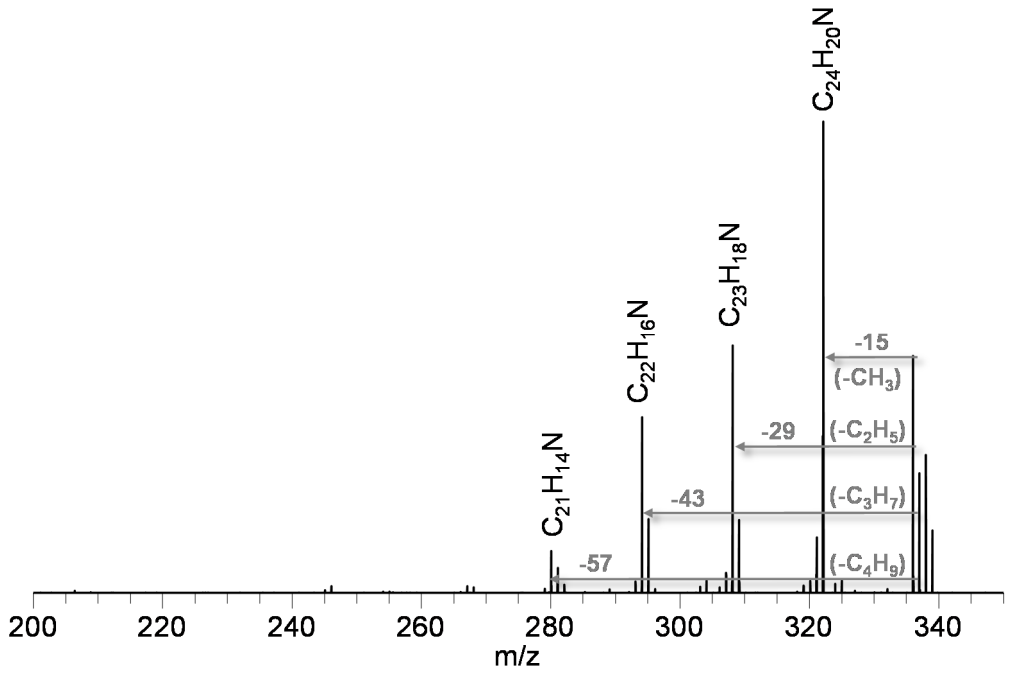

Figure 6-4. CID fragmentation mass spectrum of molecular ions of *m/z* 337 ± 1 with collision energy of 45% derived from the original asphaltene sample.

This fragmentation pattern of molecular ions of the same *m/z* using the same conditions were compared to the individual fractions derived after the two separation steps. The results show different behavior meaning different compound classes are present as the most intensive peaks as it is shown in Figure 6-5. In the first case O-containing compounds represent the most intensive fragment peaks (Figure 6-5, top) and $C_{22}H_{15}O$ is the most abundant fragment which differs from the ordinary intensity decay pattern. Furthermore, fragmentation of a hydrocarbon compound can be observed with fragments such as $C_{25}H_{21}$, $C_{24}H_{19}$, $C_{23}H_{17}$, $C_{22}H_{15}$ and $C_{21}H_{13}$. In the second fraction (Figure 6-5, center) the fragmentation of N-containing compounds can be observed as the most intensive peaks with the usual descending signals. The same

pattern can be recognized in the third case (Figure 6-5, bottom) where fragments of N, ON and N_2 heteroatom-containing compounds are present.

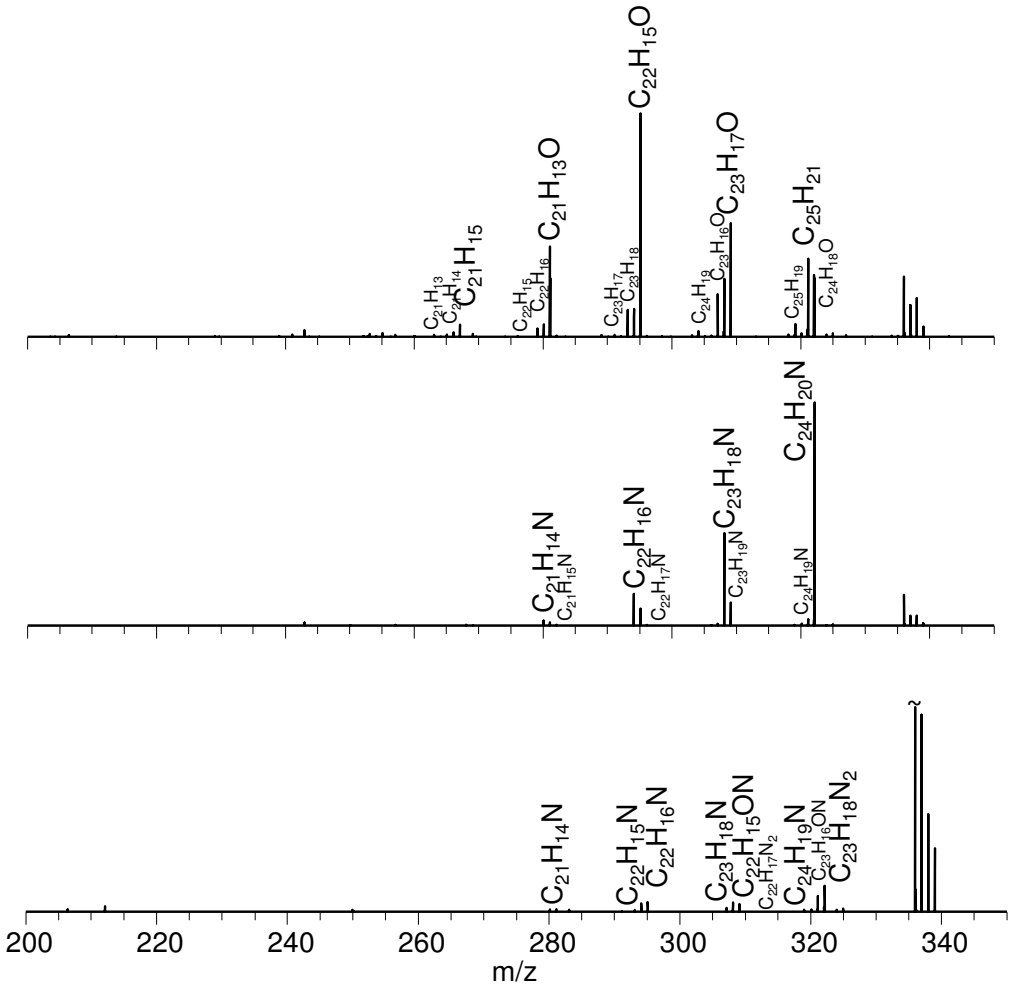

Figure 6-5. CID fragmentation mass spectra of molecular ions of *m/z* 337 ± 1 with collision energy of 45% derived from different fractions of asphaltenes after the two separation methods.

From all of this information the general structure characteristics and some possible structure can be proposed for asphaltenes. Relatively high energy was needed in order to get fragment peaks which might indicate the compact and not easily breakable structure of the analyzed compounds. Figure 6-6A shows a possible structures for an O-containing compound with the chemical composition of $C_{25}H_{22}O$ (DBE=15) based on the above mentioned results. An aromatic core can be assumed containing 5 six-membered aromatic rings and alkyl chains with maximum 5 carbon atom. However, the fragmentation pattern refers to a mixture of isomers having the same core substituted with varying length of alkyl chains (maximum pentyl group) but most likely with a methyl and butyl judging from the propyl loss as the dominant fragmentation.

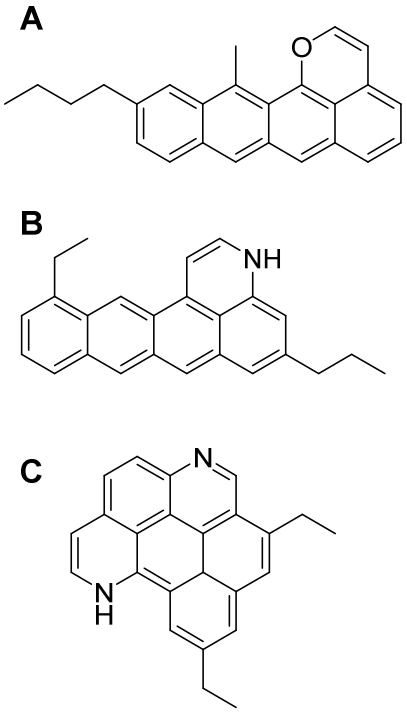

Figure 6-6. Proposed structures for asphaltene compounds containing oxygen and nitrogen (N and N_2) heteroatoms.

Similar structures – a polyaromatic core with 5-6 six-membered aromatic rings and multiple alkyl chains with various lengths ranging from 1 to 5 carbons - can be proposed for the other heteroatom-containing molecules (hydrocarbons and N, ON, N_2 class compounds) based on the most intensive peaks of the fragmentation at *m/z* 337. Figure 6-6B and Figure 6-6C represent examples for N-containing molecules (N and N_2). The only difference in the latter cases is that the dominant fragmentation was the loss of methyl group indicating the presence of ethyl groups.

6.5 Conclusion

It has been shown that detailed information of a complex mixture such as crude oil asphaltenes can be achieved by application of multidimensional chromatographic separations. The complexity is reduced here by taking advantage of the use of size exclusion chromatography followed by argentation chromatography utilizing their separation principles (molecular size as well as aromaticity plus heteroatom content). The results of the simplification together with the high-resolution tandem mass spectrometric measurement allows gaining significant and detailed structural information about compounds present in asphaltenes. The fragmentation of specific heteroatom classes within a certain aromaticity range can be investigated owing to the

separations. Therefore, advanced structural information can be achieved compared to the original asphaltenes via fragmentation of selected molecular ions. The results indicate dominant island type structures which consist of a condensed aromatic core and alkyl chains containing up to 5 carbon atoms. Similar architecture can be proposed for all kinds of heteroatom classes such as hydrocarbons, O- and N-containing compounds as the most intensive fragment peaks which can be detected only with low intensities or not at all without the application of the separation procedure.

The combination of multiple chromatographic methods followed by fragmentation experiments can be exploited in future studies for a detailed structural analysis of other crude oil fractions.

6.6 References for Chapter 6

[1] J. Beens, J. Blomberg, P. J. Schoenmakers. Proper Tuning of Comprehensive Two-Dimensional Gas Chromatography (GC×GC) to Optimize the Separation of Complex Oil Fractions. *Journal of High Resolution Chromatography* **2000**, *23*, 182-188.

[2] S. Panda, J. Andersson, W. Schrader. Mass-spectrometric analysis of complex volatile and nonvolatile crude oil components: a challenge. *Anal. Bioanal. Chem.* **2007**, *389*, 1329-1339.

[3] S. K. Panda, J. T. Andersson, W. Schrader. Characterization of Supercomplex Crude Oil Mixtures: What Is Really in There? *Angew. Chem. Int. Ed.* **2009**, *48*, 1788-1791.

[4] E. Y. Sheu. Petroleum AsphalteneProperties, Characterization, and Issues. *Energy Fuels* **2001**, *16*, 74-82.

[5] J. Speight. Handbook of Petroleum Analysis, Wiley Interscience, New York, **2001**.

[6] J. Speight. The Chemistry and Technology of Petroleum, Fifth Edition ed., Taylor & Francis Group, New York, **2014**.

[7] M. M. Boduszynski. Composition of heavy petroleums. 1. Molecular weight, hydrogen deficiency, and heteroatom concentration as a function of atmospheric equivalent boiling point up to 1400.degree.F (760.degree.C). *Energy Fuels* **1987**, *1*, 2-11.

[8] M. M. Boduszynski. Composition of Heavy Petroleums. 2. Molecular Characterization. *Energy Fuels* **1988**, *2*, 597–613.

[9] K. H. Altgelt, M. M. Boduszynski. Composition and analysis of heavy petroleum fractions. *Vol. 54*, CRC Press, **1994**.

[10] A. M. McKenna, J. M. Purcell, R. P. Rodgers, A. G. Marshall. Heavy Petroleum Composition. 1. Exhaustive Compositional Analysis of Athabasca Bitumen HVGO Distillates by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: A Definitive Test of the Boduszynski Model. *Energy Fuels* **2010**, *24*, 2929-2938.

[11] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Impact of Different Ionization Methods on the Molecular Assignments of Asphaltenes by FT-ICR Mass Spectrometry. *Anal. Chem.* **2012**, *84*, 5257-5267.

[12] B. Domon, R. Aebersold. Mass spectrometry and protein analysis. *Science (New York, N.Y.)* **2006**, *312*, 212-217.

[13] G. Chen, B. N. Pramanik, Y.-H. Liu, U. A. Mirza. Applications of LC/MS in structure identifications of small molecules and proteins in drug discovery. *J. Mass Spectrom.* **2007**, *42*, 279-287.

[14] C. A. Hughey, C. L. Hendrickson, R. P. Rodgers, A. G. Marshall. Elemental Composition Analysis of Processed and Unprocessed Diesel Fuel by Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2001**, *15*, 1186-1193.

[15] C. S. Hsu, C. L. Hendrickson, R. P. Rodgers, A. M. McKenna, A. G. Marshall. Petroleomics: advanced molecular probe for petroleum heavy ends. *J. Mass Spectrom.* **2011**, *46*, 337-343.

[16] K. Sripada, J. T. Andersson. Liquid chromatographic properties of aromatic sulfur heterocycles on a Pd(II)-containing stationary phase for petroleum analysis. *Anal. Bioanal. Chem.* **2005**, *382*, 735-741.

[17] S. K. Panda, W. Schrader, J. T. Andersson. β-Cyclodextrin as a stationary phase for the group separation of polycyclic aromatic compounds in normal-phase liquid chromatography. *J. Chromatogr. A* **2006**, *1122*, 88-96.

[18] M. Nocun, J. T. Andersson. Argentation chromatography for the separation of polycyclic aromatic compounds according to ring number. *J. Chromatogr. A* **2012**, *1219*, 47-53.

[19] L. M. Guricza, W. Schrader. New Separation Approach for Asphaltene Investigation: Argentation Chromatography Coupled with Ultrahigh-Resolution Mass Spectrometry. *Energy Fuels* **2015**, *29*, 6224-6230.

[20] S. Dong, A. M. Striegel. Size-Exclusion Chromatography of Asphaltenes: An Experimental Comparison of Commonly Used Approaches. *Chromatographia* **2013**, *76*, 725-733.

[21] S. Lababidi, S. K. Panda, J. T. Andersson, W. Schrader. Direct Coupling of Normal-Phase High-Performance Liquid Chromatography to Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for the Characterization of Crude Oil. *Anal. Chem.* **2013**, *85*, 9478-9485.

[22] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Characterization of Saturates, Aromatics, Resins, and Asphaltenes Heavy Crude Oil Fractions by Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy Fuels* **2012**, *26*, 3481-3487.

[23] Y. Cho, Y. H. Kim, S. Kim. Planar Limit-Assisted Structural Interpretation of Saturates/Aromatics/Resins/Asphaltenes Fractionated Crude Oil Compounds Observed by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Anal. Chem.* **2011**, *83*, 6068-6073.

[24] O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall. Asphaltenes, Heavy Oils, and Petroleomics, Springer, New York, **2007**.

[25] O. C. Mullins. The Modified Yen Model. *Energy Fuels* **2010**, *24*, 2179-2207.

[26] O. C. Mullins. Review of the Molecular Structure and Aggregation of Asphaltenes and Petroleomics. *SPE-95801-PA* **2008**, *13*, 48-57.

[27] L. M. Guricza, W. Schrader. Electrospray ionization for determination of non-polar polyaromatic hydrocarbons and polyaromatic heterocycles in heavy crude oil asphaltenes. *J. Mass Spectrom.* **2015**, *50*, 549-557.

[28] D. Borton, D. S. Pinkston, M. R. Hurt, X. Tan, K. Azyat, A. Scherer, R. Tykwinski, M. Gray, K. Qian, H. I. Kenttämaa. Molecular Structures of Asphaltenes Based on the Dissociation Reactions of Their Ions in Mass Spectrometry. *Energy Fuels* **2010**, *24*, 5548-5559.

[29] T. M. Jarrell, C. Jin, J. S. Riedeman, B. C. Owen, X. Tan, A. Scherer, R. R. Tykwinski, M. R. Gray, P. Slater, H. I. Kenttämaa. Elucidation of structural information achievable for asphaltenes via collision-activated dissociation of their molecular ions in MSn experiments: A model compound study. *Fuel* **2014**, *133*, 106-114.

[30] H. Sabbah, A. L. Morrow, A. E. Pomerantz, R. N. Zare. Evidence for Island Structures as the Dominant Architecture of Asphaltenes. *Energy Fuels* **2011**, *25*, 1597-1604.

[31] O. C. Mullins, H. Sabbah, J. Eyssautier, A. E. Pomerantz, L. Barré, A. B. Andrews, Y. Ruiz-Morales, F. Mostowfi, R. McFarlane, L. Goual, R. Lepkowicz, T. Cooper, J. Orbulescu, R. M. Leblanc, J. Edwards, R. N. Zare. Advances in Asphaltene Science and the Yen–Mullins Model. *Energy Fuels* **2012**, *26*, 3986-4003.

CHAPTER 7.

CHALLENGES IN STRUCTURE DETERMINATION OF CRUDE OIL ASPHALTENES AND MALTENES: LOOKING INTO THE DETAILS WITH HIGH-RESOLUTION MASS SPECTROMETRY

Redrafted from "L. M. Guricza, W. Schrader. Challenges in structure determination of crude oil asphaltenes and maltenes: looking into the details with high-resolution mass spectrometry", will be submitted to Energy & Fuels.

7.1 Abstract

The molecular architecture of the heavy crude oil asphaltenes is one of the central issues nowadays. The knowledge of the dominant structure type of this complex mixture is essential which makes it possible to predict their properties and behavior. Tandem mass spectrometry together with high resolution and high mass accuracy is the only method that enables the analysis of individual component structures of such a complex mixture. The fragmentation of individual molecules can be tracked and analyzed in detail due to the applied simplification procedure. Here we demonstrate the structural differences between asphaltenes and maltenes via fragmentation experiments. The results indicate that the dominant structure of asphaltenes obtained from a heavy crude oil is island-type: one large condensed core with only a few short alkyl side chains. However, experimental evidence was also found for the presence of archipelago-type structures (fragment peaks in the lower mass range refer to the loss of aromatic moieties) corresponding to the continuum of carbon space. Maltenes contain smaller aromatic core substituted with long aliphatic side chains. Based on the results some possible structures can be proposed.

7.2 Introduction

Asphaltenes is the crude oil fraction which is insoluble in paraffinic solvents such as *n*-heptane and soluble in toluene or benzene. Regarding the composition, it is a very complex mixture of highly condensed polyaromatic hydrocarbons substituted with alkyl chains with the presence of heteroatoms (N, O, S) and metals.[1] It is necessary to explore them due to the need of constantly increasing energy demand. Therefore the heavy, more viscous unconventional crude oil sources are becoming more emphatic as they can be upgraded into petroleum. Additionally, asphaltenes have found broad applications as row materials in road construction.[2, 3] However, asphaltenes are quite problematic in petroleum industry since they likely occlude pipes during oil transportation and separation and poison catalyst during refining processes.[4] In order to avoid the undesirable phenomena, their thorough investigation is required. Predicting the properties and behavior demands the knowledge of detailed structural composition.

While the exploration of the molecular weight distribution of asphaltenes is well accomplished [5, 6], there are still uncertainties in determination of their molecular architecture and dominating structures and it needs a better understanding. Two types of structural models have been proposed, namely island-type model and archipelago model.[1, 7] According to the island model the molecules consist of one polycondensed core with external aliphatic chains.[7-10] Archipelago model suggests that several fused moieties are bridged together via aliphatic chains.[11] In both cases of proposed structures saturated rings may also be part of the fused system.[12] In the literature evidences can be found for both kinds of structures based on different methods and measurements. However, the fundamental statement according to Yen-Mullins model is that the island molecular architecture dominates.[10] Nevertheless, the structure type and also other properties can highly depend on the origin of the sample. Hurt and co-workers have demonstrated that molecules in coal asphaltenes and in petroleum-derived asphaltenes have shown significantly different results regarding the length of alkyl chains and the size of aromatic cores.[13] Therefore investigations are needed in each individual sample.

For exploration of molecular structure of a complex mixture, tandem mass spectrometry is a suitable method of choice. This method is very popular for investigation of the structure of individual molecules, however in case of a mixture the

problem to be solved is slightly more complicated due to the high amount of fragment peaks. In the literature, some studies can be found about the investigation of fragmentation of asphaltene-like standard compounds and the differences in fragmentation pattern between island and archipelago model compounds are noticeable and easily distinguishable.[12, 14-17] Island-type molecules lose their alkyl chains via α-cleavage or only hydrogen which demands relatively high energy. Molecules with archipelago structures have intensive fragment ions at the *m/z* values of individual cores. Tandem mass spectrometry seems to be a very promising method, however, the exact interpretation of the fragmentation pathways of large aromatic compounds are not totally clear and requires deeper investigations with standard model molecules.

In this study the fragmentation behavior was investigated via collision-induced dissociation (CID). After isolation of the target compounds within the selected mass window, structure analysis can be accomplished using different activation energies. However, in case of the complex asphaltenes, the isolation of only one compound is impossible, because within the selected mass window (usually 1 Da) we have to deal with more than 100 different ions.[18] Therefore, the analysis on a molecular level requires high mass accuracy and high mass resolution. Additionally, simplification methods such as chromatographic separations need to be involved in order to reduce the complexity therefore the amount of different species.

The purpose of our investigation here was to find out which model can describe the dominant structure of asphaltene molecules derived from heavy crude oil better. On the other hand, the fragmentation pattern of asphaltenes was compared with maltenes in order to get a deeper insight into the structure types of crude oil fractions and to experimentally prove the differences via fragmentation measurements. We want to provide experimental confirmation that fairly different compounds can be detected in asphaltenes and maltenes, in general compounds with higher DBE for asphaltenes. Additionally, based on our investigations we try to propose some possible occurring structures in both samples.

7.3 Experimental section

7.3.1 Sample preparation

A heavy crude oil from a North American source was used in this study. Asphaltenes were prepared using *n*-heptane (HPLC grade, Merck, Germany) for precipitation according to a modified IP 143 method.[19] Maltenes is the *n*-heptane-soluble fraction which was concentrated for further measurements. For direct infusion in APPI measurements, the solid asphaltene sample was sonicated for 20 minutes in a mixture of toluene and chloroform (7:3 v/v), diluted to a final concentration of 250 µg/ml and then analyzed. Maltenes were diluted using also a mixture of toluene and chloroform (7:3 v/v) to the same final concentration without any further treatment. For electrospray measurements dichloromethane and methanol (1:1 v/v) was used as a solvent mixture.

For sample simplification in case of both samples (asphaltenes and maltenes), two chromatographic separations were applied consecutively with fraction collection after a certain time interval. First size exclusion chromatography was performed on an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany). A combination of styrene-divinyl benzene copolymer analytical columns (300 x 8.0 mm ID; 5 µm particle size; 1000 and 100 Å pore sizes, respectively) (PSS, Mainz, Germany) was used as stationary phase and THF as mobile phase at a flow rate of 1 ml/min. The fraction collection was accomplished with a sampling window of 30 seconds. The resulting samples were separated thereafter with argentation chromatography using a silver(I)-mercaptopropyl silica gel stationary phase synthesized in our laboratory.[20] The measurement was performed on an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Bremen, Germany). In case of asphaltenes, a mixture of toluene and chloroform (7:3 v/v) was used as mobile phase at a flow rate of 2 ml/min. A stepwise increasing amount of dimethyl sulfoxide (DMSO) was applied according to the following: 0% (0-10 min), 2% (10-20 min), 5% (20-30 min), 10% (30-40 min), 15% (40-45 min), 20% (45-50 min) and returned to 0% (50-60 min).[21] The fractions were collected within each step. In case of maltenes the mobile phase was different, a mixture of cyclohexane and chloroform (7:3 v/v) with stepwise increasing amount of isopropanol (0, 0.5, 2, 5, and 10% after each 12 min interval).

7.3.2 Mass spectrometry and data analysis

Mass analysis was performed on a research-type Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a standard ESI and APPI source. Data were collected and processed with LTQ FT Ultra 2.5.5 (Thermo Fisher Scientific, Bremen, Germany) system in a mass range of 200-900 (full scan) at a mass resolving power of 480 000 (FWHM at *m/z* 400) in positive mode. In case of electrospray ionization, the sample solutions were infused at a flow rate of 5 µl/min and the ionization was accomplished with a stainless steel capillary using 4 kV needle voltage. For the atmospheric pressure photoionization measurement, a Syagen Krypton VUV lamp (Syagen Technologies, Tustin, CA, USA) was used with photon emission at 10.0 and 10.6 eV. The flow rate was 10 µl/min, the thermal nebulizer was set to 350 °C and the nitrogen sheath gas flow was 20 (arbitrary unit).

For MS/MS measurements some randomly selected molecular ions were isolated with an isolation window width of 1.0 Da. The isolated ions were kinetically excited at the value of 30% and 45% (in case of Thermo Scientific LTQ instruments it is called normalized collision energy) using collision-induced dissociation (CID) with helium as collision gas.

The acquired data were analyzed by Xcalibur 2.2 software (Thermo Fischer Scientific, Bremen, Germany). The applied parameters and chemical constraints for the peak assignments were the followings: $0 < H < 1000$, $0 < C < 200$, $0 < N < 3$, $0 < O < 3$, $0 < S < 3$, $0 < DBE < 40$ with a maximum mass error of 1 ppm.

Double bond equivalent (DBE) is an important parameter which refers to the sum of the rings and double bonds within a molecule and used to compare the unsaturation states and aromaticity. For a given molecular formula ($C_cH_hN_nO_oS_s$) it is calculated as $DBE = c - \frac{h}{2} + \frac{n}{2} + 1$.

7.4 Results and discussion

7.4.1 Characterization of heavy crude oil asphaltenes and maltenes

High-resolution mass spectrometry allows a detailed characterization of crude oil as it is shown in Figure 7-1. In the mass spectra of the *n*-heptane-soluble fraction of heavy crude oil (maltenes, Figure 7-1A) and the *n*-heptane-insoluble fraction (asphaltenes,

Figure 7-1B) each individual signal represent a chemical composition incorporating several isomers. The detected compounds can be sorted by the amount of heteroatom classes and by the type of detected species, protonated (labelled as [H]) or radical ions. The difference between asphaltenes and maltenes can be already observed by comparing the most abundant heteroatom classes. Asphaltenes contain relatively high amount of different heteroatom classes such as N-containing compounds, while hydrocarbons are the most dominant for maltenes (Figure 7-1, A and B center). Additionally, another illustration in Figure 7-1 (A and B bottom) shows the obvious differences where hydrogen to carbon atom ratio (H/C) is plotted against *m/z* calculated for all detected species in the mass spectra. While H/C range for maltenes is between 0.5 and 2 and the majority of the assignments are above 1.0 in the mass range of 400-800, for asphaltenes this H/C ratio falls in the range of 0.4-1.4. This information provides a hint about the overall aromaticity of the sample. However, further characteristics of the individual compounds such as structural differences between asphaltenes and maltenes cannot be gained without further fragmentation experiments.

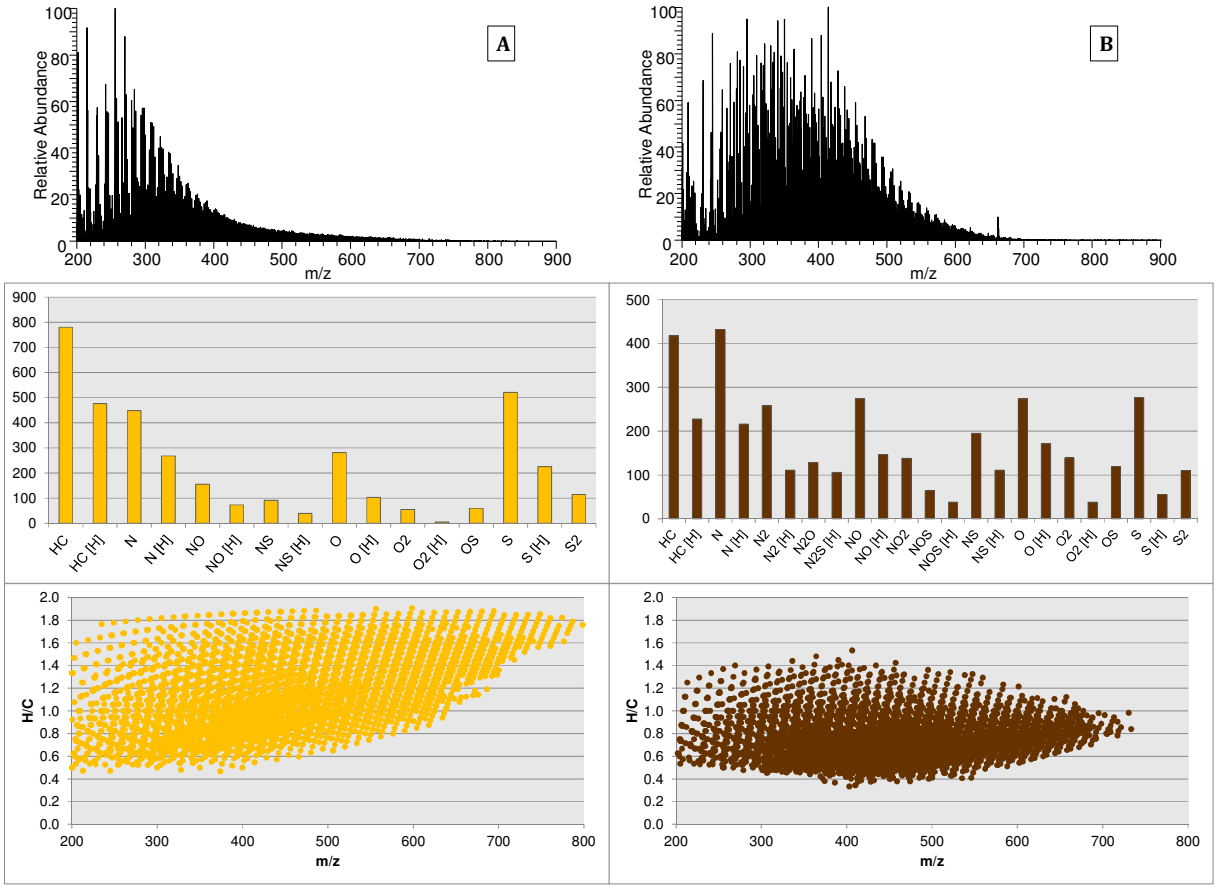

Figure 7-1. Comparison of APPI+ mass spectra in full scan mode (top), class distribution of the main detected compounds (center) and H/C versus *m/z* (bottom) in case of maltenes (A) and asphaltenes (B).

7.4.2 Comparison of fragmentation behavior of asphaltenes and maltenes

In this study the main focus was on the investigation of structural characteristics of asphaltenes and the results were compared to structural data obtained from maltenes. In both cases CID mass spectra are presented for ions of *m/z* 422 with ESI (Figure 7-2, A and C) and APPI ionization technique (Figure 7-2, B and D).

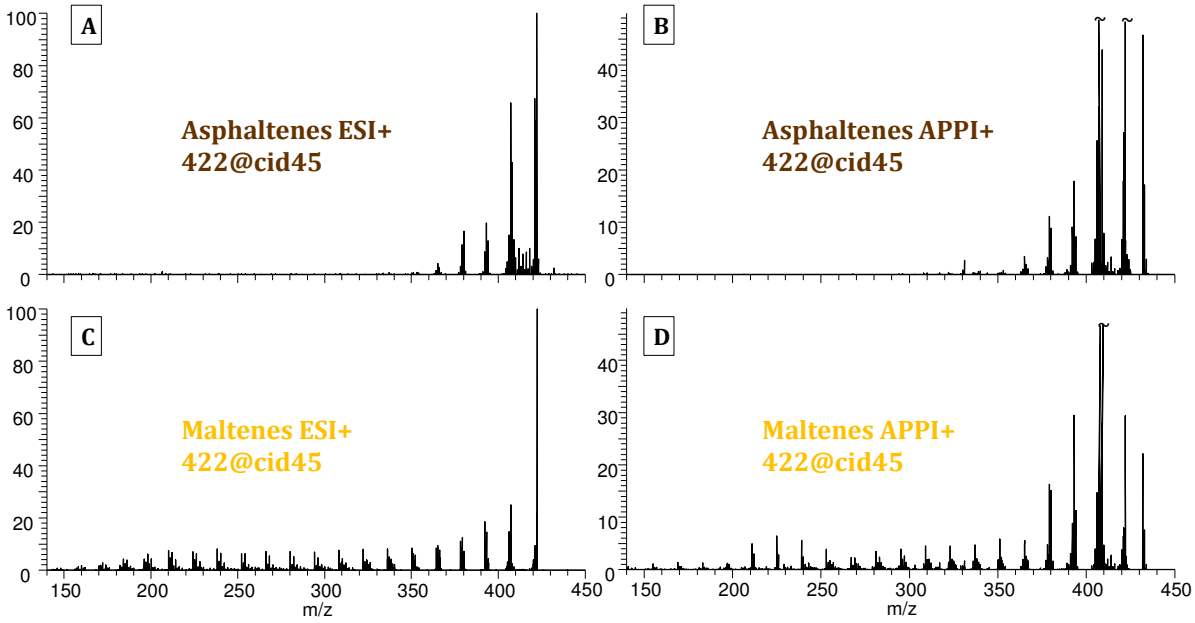

Figure 7-2. CID mass spectra of asphaltenes and maltenes measured for ions of *m/z* 422 with ESI (A and C) and APPI ionization technique (B and D).

In maltenes, CID mass spectra both from ESI and APPI ionization (Figure 7-2, C and D) resulted in much higher number of fragment peaks especially in the low mass range (*m/z* 150-300). These fragment peaks below *m/z* 300 are absent in case of asphaltenes. More fragment peaks for maltenes mean that more fragile structure is present which indicates fairly different structures overall. For instance, the number of aromatic and aliphatic carbon atoms therefore the number of alkyl chains and aromatic rings can be significantly different in maltenes compared to asphaltenes. This important difference is reflected already in the number of H atoms relative to C atoms (Figure 7-1, bottom). Since the overall H/C ratio for maltenes is higher compared to asphaltenes, higher amount of saturated carbon atoms therefore more alkyl chains can be expected.

However, this representation gives only general idea about the differences. In order to be able to do specific observations we need to look into the details on the individual peaks and fragmentations of selected mass windows. For structure determination it is

necessary to simplify the complex sample otherwise the fragmentation provides extremely complicated spectra with all of the fragment peaks from the selected mass window making the evaluation impossible. In this study two chromatographic separations were applied consecutively using fraction collections where the fractions of the first separation were injected onto the column of the second separation method. The first separation method is size exclusion chromatography where the separation is based on the molecular size while in the second dimension a separation using argentation chromatography was implemented where molecules are separated based on their aromaticity and heteroatom content.[21] After the application of these simplification steps the resulting samples are certainly containing fewer amounts of different species resulting in a more simplified fragmentation pattern compared to the original. This allows gaining structural information about much less complex samples leading structural information of individual ions.

The differences between the isolated compounds of simplified asphaltene and maltene samples at *m/z* 337 and 422 are shown in Figure 7-3.

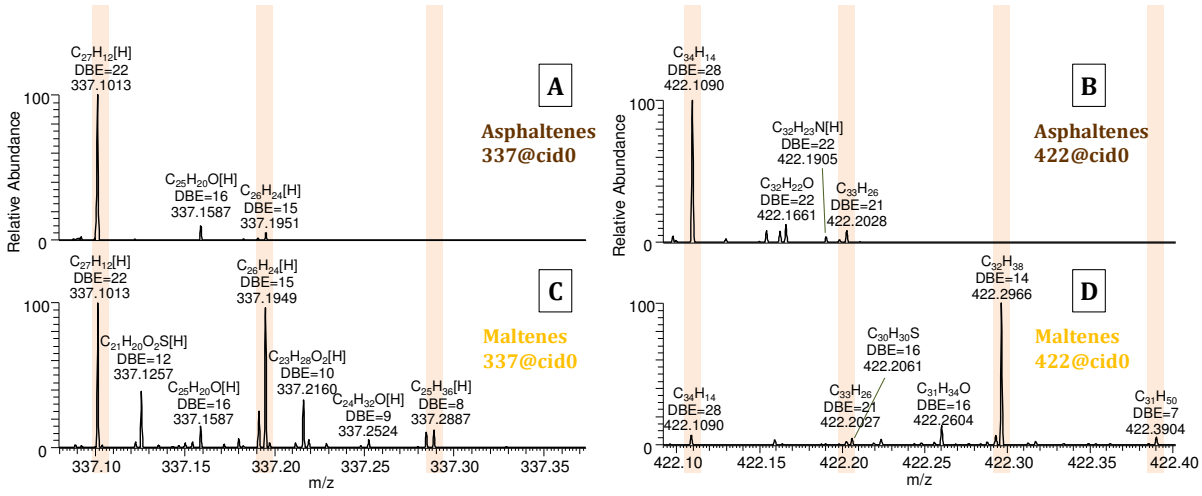

Figure 7-3. Comparison of isolated compounds of asphaltenes and maltenes at *m/z* 337 (A and C) and 422 (B and D).

Within the selected mass windows compounds with various DBE are present. For a given compound class (e.g. hydrocarbons, marked with red in Figure 7-3) the DBE differences within one nominal mass are always 7. This is expected because compounds within the same nominal mass can differ in their compositions with +1 C and –12 H atom resulting in DBE difference of 7 based on the equation definition. For example at *m/z* 422 $C_{31}H_{50}$ (DBE=7), $C_{32}H_{38}$ (DBE=14), $C_{33}H_{26}$ (DBE=21) and $C_{34}H_{14}$ (DBE=28) can be

detected (ΔDBE is always 7). It is obvious from Figure 7-3D that in case of maltenes at selected nominal mass compounds with lower DBE can be observed more intensively at around e.g. 422.30, while these peaks with compounds having low DBE are not present in case of asphaltenes, but other signals at *m/z* 422.10-422.20 instead are present with higher intensity. Therefore, in fragmentation spectra peaks with higher DBE can be expected for asphaltenes and lower DBE for maltenes. This is kind of obvious, but here we demonstrate the clear experimental confirmation.

7.4.3 Structure evaluation of asphaltenes

During the fragmentation experiments of asphaltenes compounds, after isolation of the targeted ions, relatively high energy (45%) was needed in order to get fragmentation which may indicate the massive and not easily fragile structure of the analyzed asphaltene components. After isolation of selected mass windows, in the fragmentation spectra the mass differences of the main intensive peaks are 15 and 14 Da which is in good agreement with previously presented results.[13, 15] These differences refer to the loss of alkyl chains (-CH_3, -C_2H_5, -C_3H_7, -C_4H_9, etc.). Having two peaks with a difference of exactly 14.01565 Da means a difference of a CH_2 group. However, in a complex mixture where several compounds are fragmented at the same time, we are talking about “peak groups” which need to be analyzed in more detail. Each peak is responsible for one loss of alkyl chain; therefore the length of the alkyl chain present on the molecules can be determined. As Figure 7-4 shows the example of the CID mass spectrum of ions at *m/z* 422, the number of fragment ion series is only a few (up to 6), so there is only a few different alkyl chain (max. 5-6 carbon atom long) present on the targeted molecules. This shows similarities with the fragmentation results from coal asphaltenes.[13] The lowest peak in the fragmented peak series indicates the size of the aromatic core (plus one methyl group due to the α-cleavage) of the corresponding molecules since no breakage of the central core can be expected under the applied circumstances.[15] If the molecule contains substituted benzene rings, the major cleavage is expected to be α-cleavage (or benzylic bond cleavage) generating the benzyl carbocation, which rearranges to form the so called tropylium ion as the thermodynamically most stable isomer.[22] This α-cleavage can be followed by hydrogen rearrangements producing a group of signals of each fragment with a mass difference of 1.007825 Da which makes the evaluation more complicated especially in

complex mixtures. In case of highly aromatic systems this benzylic bond cleavage occurs predominantly in resulting the tropylium-like ion (as the most stable isomer of the aromatic carbocation) by losing alkyl side chain as an alkyl radical.[12] After all, the main important information that we can get from such a fragmentation experiment is the maximum total carbon number of alkyl chains present in the molecule, furthermore the possible smallest core size.

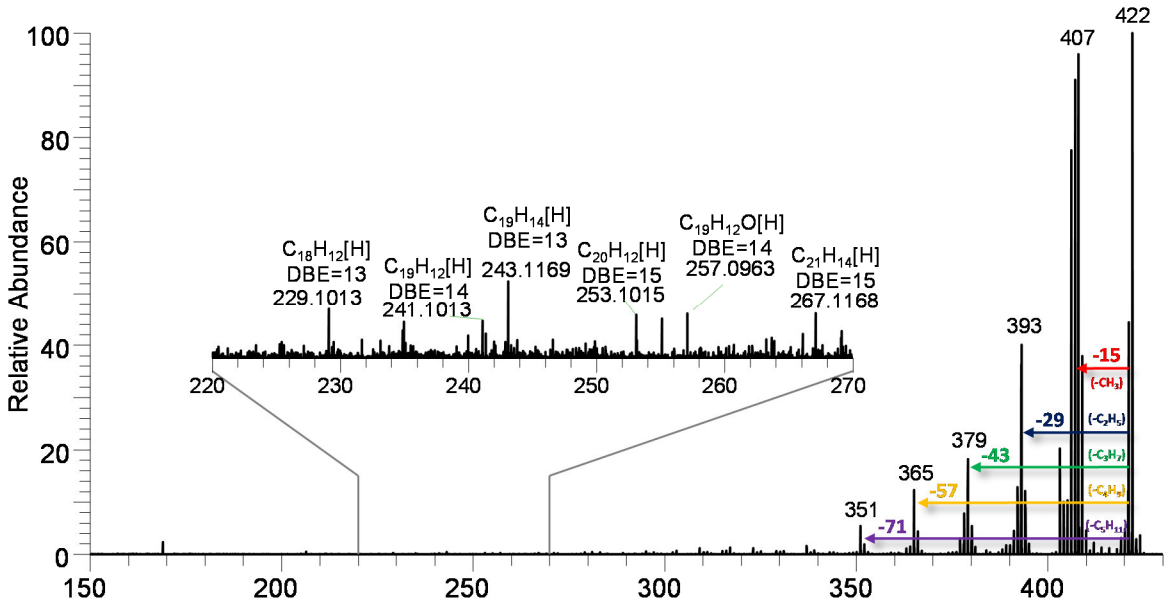

Figure 7-4. CID mass spectrum of asphaltenes measured for ions of *m/z* 422 with an energy of 45% with a zoomed-in mass range between *m/z* 220 and 270.

Considering the above mentioned observations here we found that the asphaltene components have a relatively large core structure containing 7-8 aromatic fused rings (judging from the last intensive fragment peak at *m/z* ca. 351) together with several but not too many alkyl chains present meaning that a relatively low number of isomers is present. It is worth mentioning that the fragmentation pattern was very similar at other selected ion masses. This corresponds to the island architecture molecules as the dominant structure type. Owing to the MS/MS measurements with high resolution and high mass accuracy, the isolated peaks and the corresponding fragment peaks can be followed across the whole fragmentation very accurately. From the information of the elemental composition of the isolated molecules and the core molecules, furthermore from the number of alkyl chains and chain length some possible structures (one possible isomer out of numerous possibilities) are proposed in Figure 7-5 based on the fragmentation of ions at *m/z* 422.

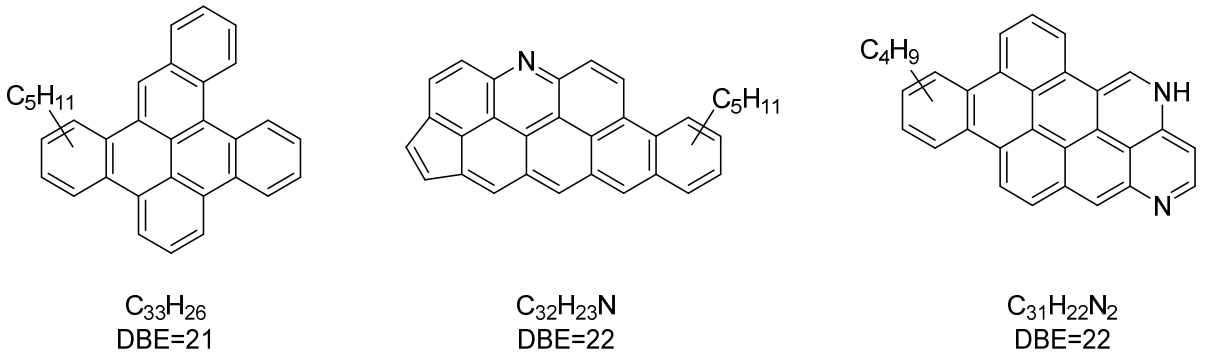

Figure 7-5. Proposed structures for asphaltenes based on fragmentation measurements at *m/z* 422.

For comprehensive analysis, the fragmentation behavior of asphaltenes was investigated by using electrospray ionization, as well. The results in this case regarding the fragmentation pattern were very similar to the results from atmospheric pressure photoionization measurements. The small number of fragment peaks and peak groups with relatively high energy imply the island architecture with only a few short alkyl chains.

Although, it was found that island-type molecules are dominant, in case of fragmentation of the most simplified asphaltene samples in the low *m/z* ranges fragment peaks with relatively low DBE (13-15) are present as well, however with fairly low intensity (Figure 7-4, zoomed-in spectrum). The observed DBE values were lower than the precursor ions have in the isolation window. This refers to the loss of aromatic moieties which would be expected for the fragmentation of archipelago-type compounds.[15] This result can give us a hint that archipelago structures cannot be completely excluded. Keeping in mind the experiments of Borton and co-workers about the fragmentation of archipelago model molecules, the ethylene bridge is very probable to be present because it is quite fragile.[15] On the other hand, according to Groenzin and co-workers asphaltene structures may contain also saturated rings.[7] Therefore, a possible explanation for $C_{19}H_{14}[H]$ (*m/z* 243.117) protonated fragment (as the highest in the selected zoomed-in window in Figure 7-4) is presented as an example for the presence of an archipelago structure of composition $C_{33}H_{26}$ in Figure 7-6. Based on this example we could assume that the formation of tropylium-like ions takes place not only in island but also in archipelago structures. The presence of a weak signal of the other connecting ring, $C_{14}H_{10}[H]$ at *m/z* 179.086 can support the suggestion of this structure.

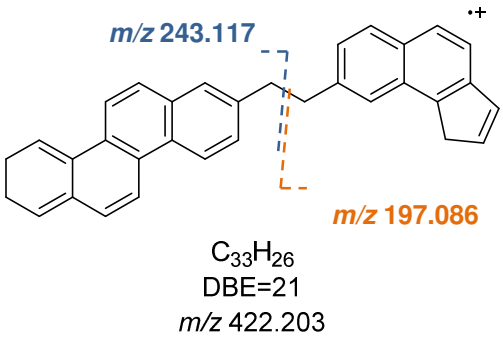

Figure 7-6. Possible archipelago-type asphaltene structure of composition $C_{33}H_{26}$.

7.4.4 Structure evaluation of maltenes

As it was already presented in Figure 7-2 (C and D), the loss of a series of alkyl chains starting from one methyl group up to 19-20 carbon atom-containing alkyl chains can be observed in case of the fragmentation of maltenes. As a consequence, unlike asphaltenes, maltenes contain long alkyl chains with varying lengths and this pattern is obviously an evidence for different isomers present at the selected mass since it is impossible for one molecule to contain so many different alkyl chains.

Peak-to-peak evaluation of the fragmentation spectrum of maltenes is way too complicated due to the numerous peaks and large number of possibilities for fragmentation pathways. Compared to asphaltenes, fragment peaks are much more abundant at low mass range as it is presented in Figure 7-7. The CID mass spectrum measured for ions at *m/z* 337 with energy of 40% and mass scale-expanded view in the mass range of 100-200 shows several hydrocarbon compounds with various DBE values (5-10).

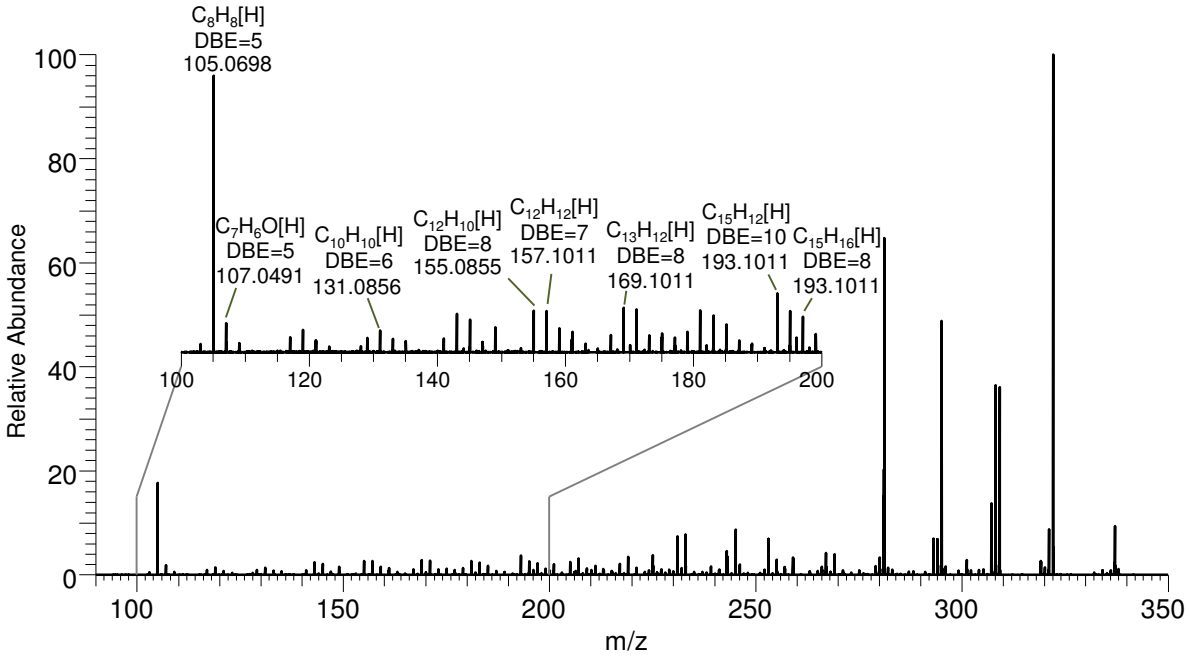

Figure 7-7. CID mass spectrum of maltenes measured for ions of *m/z* 337 with energy of 40% with a zoomed-in mass range between *m/z* 100 and 200.

Looking back at the isolated compounds at *m/z* 337 (Figure 7-3C) hydrocarbons are detected in their protonated form with DBE of 8 ($C_{25}H_{36}[H]$), DBE of 15 ($C_{26}H_{24}[H]$) and DBE of 22 ($C_{27}H_{12}[H]$). With energy of 40% in the fragmentation pattern $C_{27}H_{12}[H]$ did not show any fragmentation. Of the series of the ion $C_{26}H_{24}[H]$ with a DBE of 15 only a few fragments were observed. In this series $C_{21}H_{14}$ is the smallest fragment peak therefore $C_{20}H_{12}$ is assumed to be the core of this ion. Figure 7-8A shows possibility for this parent hydrocarbon molecule with a DBE of 15.

Most of the hydrocarbon fragment peaks could be originated from the parent molecule with DBE of 8 ($C_{25}H_{36}[H]$) by the losses of different length of alkyl chains (therefore DBE value remains the same). Due to the broader DBE range of fragment peaks in the low mass range hydrogen rearrangements might be assumed.[15] Some possibilities for this kind of structure (DBE=8 with long alkyl side chain) is presented in Figure 7-8B. From these suggested structures biphenyl is one of the most interesting. Although, it does not have a fused core, the strong aryl-aryl carbon-carbon bond makes it very stable. The bond dissociation energy in biphenyl molecules is larger (418 kJ/mol) than the energy needs for α-cleavage in e.g. ethylbenzol (301 kJ/mol).[23] Its stability allows us to presume the presence of different biphenyl isomers.

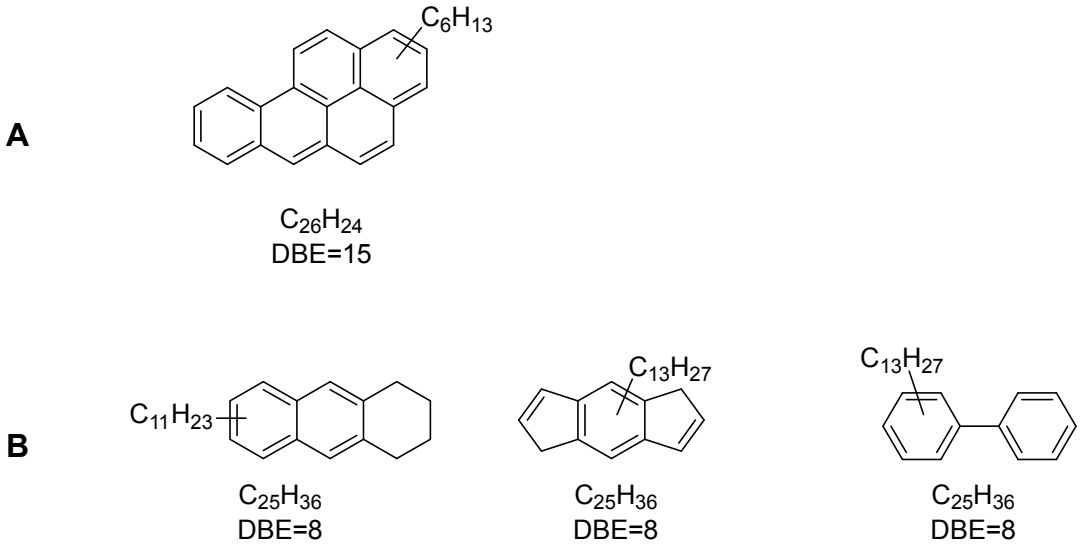

Figure 7-8. Proposed structures for maltenes based on fragmentation measurements at *m/z* 337.

7.5 Conclusion

In has been shown that tandem mass spectrometry with high resolution and high mass accuracy is a good method of choice for structure elucidation of simplified complex mixtures. The differences of fragmentation behavior of maltenes and asphaltenes are remarkable which was demonstrated in this study. These experiments can give us information on a molecular level and with detailed evaluation of the fragmentation pattern some possible structures can be proposed both for asphaltenes and maltenes. Island-type structures were found to be the dominant in case of asphaltenes from a heavy crude oil containing one condensed core with 7-8 fused rings substituted with few short 5-6 carbon atom long alkyl chains. However, experimental evidence for some archipelago-type structures (fragment peaks in the lower mass range and with low DBE) due to the high degree of simplification was also observed. Based on the fragmentation of maltenes, the majority of these compounds contain smaller ring sizes together with long alkyl side chains containing up to 19-20 carbon atoms.

Nevertheless, in order to fully understand the fragmentation pathways and mechanisms of the individual components, various labelled and/or multiply labelled isotopomers would be needed.

7.6 References for Chapter 7

[1] O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall. Asphaltenes, Heavy Oils, and Petroleomics, Springer, New York, **2007**.

[2] M. R. Gray. Consistency of Asphaltene Chemical Structures with Pyrolysis and Coking Behavior. *Energy Fuels* **2003**, *17*, 1566-1569.

[3] T. F. Yen, G. V. Chilingarian. Asphaltenes and Asphalts, 2, Elsevier Science B.V, Amsterdam, The Netherlands, **2000**.

[4] E. Y. Sheu. Petroleum AsphalteneProperties, Characterization, and Issues. *Energy Fuels* **2001**, *16*, 74-82.

[5] O. C. Mullins. The Modified Yen Model. *Energy Fuels* **2010**, *24*, 2179-2207.

[6] A. E. Pomerantz, M. R. Hammond, A. L. Morrow, O. C. Mullins, R. N. Zare. Asphaltene Molecular-Mass Distribution Determined by Two-Step Laser Mass Spectrometry. *Energy Fuels* **2009**, *23*, 1162-1168.

[7] H. Groenzin, O. C. Mullins. Molecular Size and Structure of Asphaltenes from Various Sources. *Energy Fuels* **2000**, *14*, 677-684.

[8] A. B. Andrews, J. C. Edwards, A. E. Pomerantz, O. C. Mullins, D. Nordlund, K. Norinaga. Comparison of Coal-Derived and Petroleum Asphaltenes by 13C Nuclear Magnetic Resonance, DEPT, and XRS. *Energy Fuels* **2011**, *25*, 3068-3076.

[9] O. C. Mullins, B. Martínez-Haya, A. G. Marshall. Contrasting Perspective on Asphaltene Molecular Weight. This Comment vs the Overview of A. A. Herod, K. D. Bartle, and R. Kandiyoti. *Energy Fuels* **2008**, *22*, 1765-1773.

[10] O. C. Mullins, H. Sabbah, J. Eyssautier, A. E. Pomerantz, L. Barré, A. B. Andrews, Y. Ruiz-Morales, F. Mostowfi, R. McFarlane, L. Goual, R. Lepkowicz, T. Cooper, J. Orbulescu, R. M. Leblanc, J. Edwards, R. N. Zare. Advances in Asphaltene Science and the Yen–Mullins Model. *Energy Fuels* **2012**, *26*, 3986-4003.

[11] A. H. Alshareef, A. Scherer, X. Tan, K. Azyat, J. M. Stryker, R. R. Tykwinski, M. R. Gray. Formation of Archipelago Structures during Thermal Cracking Implicates a Chemical Mechanism for the Formation of Petroleum Asphaltenes. *Energy Fuels* **2011**, *25*, 2130-2136.

[12] T. M. Jarrell, C. Jin, J. S. Riedeman, B. C. Owen, X. Tan, A. Scherer, R. R. Tykwinski, M. R. Gray, P. Slater, H. I. Kenttämaa. Elucidation of structural information achievable for asphaltenes via collision-activated dissociation of their molecular ions in MSn experiments: A model compound study. *Fuel* **2014**, *133*, 106-114.

[13] M. R. Hurt, D. J. Borton, H. J. Choi, H. I. Kenttämaa. Comparison of the Structures of Molecules in Coal and Petroleum Asphaltenes by Using Mass Spectrometry. *Energy Fuels* **2013**, *27*, 3653-3658.

[14] H. Sabbah, A. L. Morrow, A. E. Pomerantz, O. C. Mullins, X. Tan, M. R. Gray, K. Azyat, R. R. Tykwinski, R. N. Zare. Comparing Laser Desorption/Laser Ionization Mass Spectra of Asphaltenes and Model Compounds. *Energy Fuels* **2010**, *24*, 3589-3594.

[15] D. Borton, D. S. Pinkston, M. R. Hurt, X. Tan, K. Azyat, A. Scherer, R. Tykwinski, M. Gray, K. Qian, H. I. Kenttämaa. Molecular Structures of Asphaltenes Based on the Dissociation Reactions of Their Ions in Mass Spectrometry. *Energy Fuels* **2010**, *24*, 5548-5559.

[16] H. Sabbah, A. L. Morrow, A. E. Pomerantz, R. N. Zare. Evidence for Island Structures as the Dominant Architecture of Asphaltenes. *Energy Fuels* **2011**, *25*, 1597-1604.

[17] W. Tang, M. R. Hurt, H. Sheng, J. S. Riedeman, D. J. Borton, P. Slater, H. I. Kenttämaa. Structural Comparison of Asphaltenes of Different Origins Using Multi-stage Tandem Mass Spectrometry. *Energy Fuels* **2015**, *29*, 1309-1314.
[18] A. Gaspar, E. Zellermann, S. Lababidi, J. Reece, W. Schrader. Impact of Different Ionization Methods on the Molecular Assignments of Asphaltenes by FT-ICR Mass Spectrometry. *Anal. Chem.* **2012**, *84*, 5257-5267.
[19] L. M. Guricza, W. Schrader. Electrospray ionization for determination of non-polar polyaromatic hydrocarbons and polyaromatic heterocycles in heavy crude oil asphaltenes. *J. Mass Spectrom.* **2015**, *50*, 549-557.
[20] M. Nocun, J. T. Andersson. Argentation chromatography for the separation of polycyclic aromatic compounds according to ring number. *J. Chromatogr. A* **2012**, *1219*, 47-53.
[21] L. M. Guricza, W. Schrader. New Separation Approach for Asphaltene Investigation: Argentation Chromatography Coupled with Ultrahigh-Resolution Mass Spectrometry. *Energy Fuels* **2015**, *29*, 6224-6230.
[22] J. H. Gross. Mass spectrometry, Wiley Online Library, **2004**.
[23] J. A. Dean. Lange's handbook of chemistry, McGraw-Hill Professional, New York, **1998**.

CHAPTER 8.
GENERAL CONCLUSION

The objective of this study was to characterize and gain structural information of heavy crude oil, especially asphaltenes which is the most problematic fraction causing for example deposit formation and catalyst poisoning in the oil industry. A comprehensive characterization is necessary to understand their unfavorable behavior.

Mass spectrometry with high resolution and high mass accuracy using different ionization techniques is capable to provide information about individual chemical compounds even from such a complex mixture as heavy crude oil containing several thousands of different compounds.

First of all, it has been shown that although atmospheric pressure photoionization is used most frequently for the ionization of compound mixture with a broad polarity range, electrospray can be also an efficient alternative to provide a thorough characterization of such highly condensed aromatic structures. Under controlled conditions polyaromatic hydrocarbons and heterocycles can be ionized by electron transfer creating radical cations, as well. It can be assumed that an electrochemical reaction takes place at the metal/solution interface inside the electrospray capillary resulting in the formation of radical cations when no proton donor agents present. Thus the usability of electrospray can be extended and not only polar but also non-polar compounds can be analyzed in case of condensed aromatic structures such as asphaltenes.

Chromatographic separation is beneficial prior to MS to eliminate or reduce the undesirable matrix effects, space charge effects, ion discrimination, and ion suppression effects. Selection of a suitable chromatographic method is a critical point due to the fact that heavy crude oil components are not volatile enough for gas chromatographic analysis. Furthermore, in liquid chromatography the major challenge is to find a proper solvent system which is compatible with the sparse solubility of asphaltenes, reducing the available options to toluene, chloroform, dichloromethane and tetrahydrofuran. It has been shown that size exclusion chromatography is one possible option which can separate asphaltenes according to their molecular weight (while no specific compound class separation was expected). After optimization, this separation resulted in a

decreased complexity, and therefore increased information depth was achieved and new compounds could be detected. Argentation chromatography (silver(I) ions are immobilized on the silica gel surface) has been found to be another possibility for simplifying complex samples in which case the separation is based on the aromaticity, furthermore the content of heteroatoms has also an influence. This separation method was successfully applied to heavy crude oil where compounds within a higher DBE range up to 30-35 could be detected which was not observed without the application of this separation. In case of asphaltenes this method could not be directly adopted because isopropanol as competitive ligand was not strong enough to supersede asphaltenes which coordinates strongly to the stationary phase. The solvent system had to be changed and DMSO as a stronger competitive solvent was used for successful elution. It has been shown that isomeric compounds could be separated by using this approach.

In the end, structural elucidation of the individual chemical components of extremely complex mixtures was the most challenging part of this thesis. MS/MS measurements are capable of providing structural information on a molecular level, but the main issue in crude oil is that the isolation window contains up to 200 ions. Therefore, the structural analysis required the combination of multiple separation steps prior to MS to be able to gain meaningful information. The above mentioned chromatographic separations (first size exclusion chromatography then argentation chromatography) were applied consecutively to achieve sufficient simplification of heavy crude oil fractions (asphaltenes and maltenes). The resulting simplified fractions were then used for tandem mass spectrometric studies by isolating individual mass windows and gaining fragmentation of the precursor ions. The results showed that in case of asphaltenes the dominant species found are compounds with island-type structure meaning that only one large condensed aromatic core is present which contains a few short alkyl side chains. However, the presence of archipelago-type structures was also postulated based on specific fragmentation patterns. The molecular architecture of the maltene fraction was also investigated. It has been shown experimentally that compared to asphaltenes, maltenes contain considerably smaller aromatic cores substituted with much longer aliphatic side chains. In case of both fractions some possible structures could be proposed based on the mass spectrometric results. All these data lead to a

theory of how the different types of compounds within the carbon space of crude oil are made up.

APPENDIX

List of abbreviations

ACN	acetonitrile
AGC	automatic gain control
APCI	atmospheric pressure chemical ionization
API	atmospheric pressure ionization
APLI	atmospheric pressure laser ionization
APPI	atmospheric pressure photoionization
CARS	condensed aromatic ring structure
CI	chemical ionization
CID	collision-induced dissociation
DART	direct analysis in real time
DBE	double bond equivalent
DESI	desorption electrospray ionization
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EI	electron ionization
ESI	electrospray ionization
eV	electron volts
FAB	fast atom bombardment
FCS	fluorescence correlation spectroscopy
FD/FI	field desorption/field ionization
FT-ICR	Fourier transform ion cyclotrone resonance

FWHM	full width at half maximum
GC	gas chromatography
HPLC	high-performance liquid chromatography
ICP	inductively coupled plasma
ID	internal diameter
IE	ionization energy
IPA	isopropyl alcohol
IUPAC	international union of pure and applied chemistry
KMD	Kendrick mass defect
KNM	Kendrick nominal mass
kV	kilovolts
LC	liquid chromatography
LDI	laser desorption ionization
LTQ	linear trap quadrupole
LVEI	low-voltage electron ionization
m/z	mass-to-charge ratio
MALDI	matrix assisted laser desorption ionization
MeOH	methanol
mmu	milli mass unit
MS	mass spectrometry
NMR	nuclear magnetic resonance
NP	normal phase
PAC	polar amino cyano
PAH	polycyclic aromatic hydrocarbons
PANH	polycyclic aromatic nitrogen heterocycles
PASH	polycyclic aromatic sulfur heterocycles

ppb	parts per billion
ppm	parts per million
R	resolution
REMPI	resonance enhanced multiphoton ionization
RIC	reconstructed ion chromatogram
RF	radio frequency
RP	reversed phase
S/N	signal-to-noise ratio
SARA	saturates, aromatics, resins and asphaltenes
SEC	size exclusion chromatography
SIM	selected ion monitoring
THF	tetrahydrofuran
TIC	total ion chromatogram
TOF	time-of-flight
TRFD	time-resolved fluorescence depolarization
UV/Vis	ultraviolet-visible
VPO	vapor-pressure osmometry
XPS	X-ray photoelectron spectroscopy

List of publications

Publications in Peer-reviewed Journals

- L. M. Guricza, W. Schrader. Electrospray ionization for determination of non-polar polyaromatic hydrocarbons and polyaromatic heterocycles in heavy crude oil asphaltenes. *J. Mass Spectrom.* **2015**, 50, 549-557
- L. M. Guricza, W. Schrader. New separation approach for asphaltene investigation: argentation chromatography coupled with ultrahigh-resolution mass spectrometry. *Energy Fuels* **2015**, 29, 6224-6230
- T. Ghislain, L. M. Guricza, W. Schrader. Characterization of crude oil asphaltenes by coupling size exclusion chromatography directly to an ultrahigh-resolution mass spectrometer (submitted to Rapid Communications in Mass Spectrometry).
- L. M. Guricza, W. Schrader. Analysis of asphaltenes using online coupling of size exclusion chromatography and ultrahigh-resolution mass spectrometry (will be submitted to Journal of Chromatography A).
- L. M. Guricza, W. Schrader. Argentation chromatography coupled to ultrahigh-resolution mass spectrometry for effective separation of a heavy crude oil (submitted to Journal of Chromatography A).
- L. M. Guricza, W. Schrader. Combination of analytical techniques for structural elucidation of individual chemical compounds in extremely complex samples (will be submitted to Analytical Chemistry).
- L. M. Guricza, W. Schrader. Challenges in structure determination of crude oil asphaltenes and maltenes: looking into the details with high-resolution mass spectrometry (will be submitted to Energy & Fuels).

Poster and Oral Presentations

- L. M. Guricza, W. Schrader. Argentation chromatography coupled with ultrahigh-resolution mass spectrometry for effective separation of heavy crude oil. *49th German mass spectrometry society annual meeting (DGMS)*
  28 Feb. - 02 Mar. 2016, Hamburg, Germany (Poster presentation)
- L. M. Guricza, W. Schrader. New separation approach for asphaltene investigation: argentation chromatography coupled with ultrahigh-resolution mass spectrometry. *Fachgruppentreffen FTMS and high-resolution mass spectrometry 2015*
  17 – 18 Sept. 2015, Bonn, Germany (Poster presentation)
- L. M. Guricza, W. Schrader. Analysis of complex aromatic mixtures such as asphaltenes using online coupling LC/MS methods. *63rd ASMS conference on mass spectrometry and allied topics*
  31 May – 04 June 2015, St. Louis, Missouri, USA (Poster presentation)
- L. M. Guricza, W. Schrader. Study on highly aromatic complex mixtures such as crude oil asphaltenes with online and offline SEC-MS. *48th German mass spectrometry society annual meeting (DGMS)*
  01 - 04 Mar. 2015, Wuppertal, Germany (Oral presentation, organizer team)
- L. M. Guricza, W. Schrader. Compositional analysis of heavy crude oil samples using size exclusion chromatography in combination with ultrahigh-resolution mass spectrometry. *20th International mass spectrometry conference (IMSC)*
  24 - 29 Aug. 2014, Geneva, Switzerland (Poster presentation)
- L. M. Guricza, W. Schrader. Electrospray ionization for determination of non-polar polyaromatic hydrocarbons and polyaromatic heterocycles in crude oil asphaltenes. *62nd ASMS conference on mass spectrometry and allied topics*
  15 – 19 June 2014, Baltimore, Maryland, USA (Poster presentation)
- L. M. Guricza, W. Schrader. Compositional analysis of asphaltene sample using FT-ICR Mass Spectrometry with different ionization techniques. *47th German mass spectrometry society annual meeting (DGMS)*
  02 - 05 Mar. 2014, Frankfurt am Main, Germany (Poster presentation)

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